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CARDIOVASCULAR EFFECTS OF NICOTINE AND SMOKING

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CARDIOVASCULAR EFFECTS OF NICOTINE AND SMOKING*

Consulting Editor
MCKEEN CATTELL

Conference Chairman
CARL J. WIGGERS

24 Nov 67

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Part I. The Absorption and Fate of Nicotine

INTRODUCTORY REMARKS

Carl J. Wiggers
Frank E. Bunts Educational Institute, Cleveland, Ohio

It must not be expected that in publications of this sort all questions raised will be answered satisfactorily. From long experience I have learned that there are no crucial experiments. I have tried to design and execute such experiments for more than 50 years, and finally have given up the attempt. Data and interpretations can be regarded only as doubtful, probable, or reasonable, but not quite certain. Furthermore, deductions that appear to be crucial in one era become controversial again in the next, when they are reexamined in the light of new developments. Let me illustrate this point briefly by recalling the evolution of ideas concerning the mechanisms by which nicotine produces cardiovascular changes.

I studied pharmacology in 1903 under Arthur Cushny. It was near the beginning of an era when attempts were first made to explain the actions of drugs on a physiological basis. Regarding the pharmacodynamic actions of nicotine, we were guided by the epoch-making discovery of Langley in 1890 that the drug exerts an initial stimulating and subsequent paralyzing effect on ganglion cells. This seemed to account adequately for the initial bradycardia and subsequent tachycardia that followed injection of nicotine solutions into experimental animals. We had no precise idea of the way in which ganglionic stimulation and paralysis were induced, but hypothesized that the excitability of ganglion cells was reduced so far that the intensity of action potentials impinging upon ganglion cells was below threshold value.

A sequence of evidence substantiated the interpretations of Henry Dale that transjunctional excitations are mediated chemically. Thus it became increasingly evident that a more precise localization of nicotine action within ganglia would be necessary.

My recollections go back even farther. When I began the study of pharmacology, we knew the cardiovascular effects of nicotine as well as we do today. We could explain them satisfactorily by applying the available physiological knowledge of the day. The subsequent discoveries that other mechanisms can modify the direct effects of drugs on the heart and its nerves then began to complicate our simple interpretations. Thus Stewart and Rogoff showed in 1919 that nicotine liberates a powerful pressor substance from the adrenal medulla and, somewhat later, Burn and Bülbring showed that it also releases a powerful coronary vasoconstrictor substance from the neurohypophysis. The possible modifications of primary cardiac responses to nicotine by such secondary agents obviously needed to be considered.

In 1927 C. Heymans and his associates found that nicotine also activates chemoreceptors in the carotid sinus, which further modulate the reactions induced directly by the drug. In short, today the effects of smoking on the cardiovascular system requires an integration of the ganglionic, humoral, hormonal, and reflex actions. It is, of course, the purpose of this publication to help us crystallize our thinking with respect to the net effect of these various processes on the heart and circulation.

I cannot resist the expression of a final thought that may prove to be less facetious than it seems today: modern textbooks of pharmacology assert that nicotine is of no therapeutic value; it has only toxicological interest. Since a new branch of pharmacology—psychopharmacology—is developing rapidly, and since tobacco is probably the most ancient tranquilizing agent in common use, it may be necessary to revise our current attitude. Now all tranquilizing drugs have certain side effects. These may be beneficial, indifferent, or undesirable. It is one of the present day problems to determine whether the possible deleterious effect of smoking on the heart and blood vessels outweighs its stabilizing effects on psychic and emotional processes.

Let us approach the problems before us in a humble spirit, recognizing the temporal nature of our deductions, remaining aware of the areas of unenlightenment, and leaving room for doubt. The greatest lesson that science has taught is how much more there is to learn.

NICOTINE AND ITS BOTANICAL SOURCES*

R. F. Dawson, Marie L. Solt
Columbia University, New York, N. Y.

D. R. Christman
Brookhaven National Laboratory, Brookhaven, N. Y.

There is an extensive and well-documented body of information relating to the chemical properties of nicotine, to its occurrence and distribution in commercial varieties of tobacco, and to the changes that it undergoes during manufacture into smoking products. This information has been reviewed[1] many times in the past and need not be repeated here. Rather, since the theme of this monograph emphasizes the biological activity of a vegetable product in the animal body, it would seem profitable to inquire whether the circumstances surrounding the origin of the product in the vegetable world may contribute information of value in the comparative biochemical sense.

The Occurrence and Distribution of Nicotine

Nicotine has the distinction of being the most widely distributed of all the plant alkaloids. It occurs in two separate phyla of the plant kingdom, the Pteridophytes and the Spermatophytes. Among the Pteridophytes it has been isolated from species of Lycopodium and Equisetum. Among the Spermatophytes, it occurs in the Angiospermous families Erythroxylaceae (*Erythroxylum*), Asclepiadaceae (*Asclepias*), Crassulaceae (*Sedum*), and the Compositae (*Eclipta* and *Zinnia*), in addition to the familiar Solanaceae (such as *Nicotiana*, *Lycopersicum*, *Atropa*, *Datura*, *Solanum*, *Petunia*, and *Duboisia*). In the light of current concern about the presence of traces of poisonous materials in foodstuffs, we should mention that the concentrations of nicotine in tomato (*Lycopersicum*) and eggplant (*Solanum*) fruits are too low to arouse concern.

Nicotine occurs in all members of the genus *Nicotiana* thus far investigated. However, it is found in relatively low concentrations in the wild species that still survive. The two species *tabacum* and *rustica*, to which all commercial varieties of tobacco belong, possess a much higher nicotine content than the wild species, but it is well to remember that these two cultivated species have survived only through the efforts of man. In the philosophical sense, therefore, it is tempting to regard nicotine production as a genetically controlled capacity that is gradually being depreciated in the course of continuing evolution.

The above-described distribution of nicotine (and other alkaloids) in the plant kingdom coincides with the evolutionary development of the plant body adapted for growth on dry land. This plant body is characterized by differentiation of leaf, root, and stem organs, and of the necessary vascular and storage tissues to service them.

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In the laboratory it is possible to remove tissue masses from various portions of the tobacco plant body and bring them into microbially sterile culture. Interestingly, with a single anomalous exception, such tissue and free cell cultures do not produce nicotine. We have been led, therefore, to suppose that production of the alkaloid may be a concomitant of the still-obscure biochemistry of organ or tissue differentiation. Similar conclusions have been drawn from investigations of the relationship between nicotine production and growth activity in the plant body as indicated in the following section.

Nicotine Production and Growth Rates

In the early days of our work on nicotine biosynthesis, we found it necessary to develop an experimental system capable of giving substantial and reproducible yields of the alkaloid, preferably under microbially sterile conditions. Such a system was found in the excised tobacco root culture, which at that time had just been described by P. R. White[2] of The Rockefeller Institute for Medical Research at Princeton, N. J. Quite unexpectedly, this experimental system revealed the existence of a close rate-dependency of nicotine yield upon the growth of the root in culture. In the case of our usual experimental object, *Nicotiana tabacum* L. var. Turkish, the proportionality constant relating the 2 quantities is of the order of magnitude of 30 μg. of nicotine per mg. of root dry weight.[3] We have found a similar rate dependency to be true for a strain of *N. tabacum* L. var. Pennsylvania Seedleaf and, with a much lower proportionality constant, also for a strain of *Nicotiana glauca* Grah.

In the plant root, as grown in sterile culture, growth involves a broad range of cellular activities: division, volume increase, and differentiation. A small group of cells in the apex of each rootlet remains permanently embryonic. The progeny of these embryonic cells complete their divisions, enlargement, and differentiation within a short distance, measured in millimeters, posterior to the apex. This region is recognizable more or less readily as a morphological entity and is referred to simply as the root tip. The significant fact for the present discussion is that the root tip is the locus not only for growth activity but also for nicotine production. This fact is undoubtedly related to the rate dependency of the two processes.

Experiments aimed at analyzing this rate dependency have not yet given a clear picture. Applications of growth-inhibiting concentrations of the so-called plant growth hormone, 3-indolylacetic acid, appeared to inhibit cell enlargement and cell differentiation but not cell division.[3] Nicotine production was completely inhibited. The results of these and of many other experiments appear to indicate rather clearly the lack of correlation between cell division and nicotine synthesis. Both cell division and cell enlargement occur in tissue cultures and yet no nicotine is formed. Thus the correlation with nicotine formation once again would appear to rest with some aspect of tissue or organ differentiation. The puzzling fact is, however, that both tissue and organ differentiation occur in stems and leaves. However, the alkaloid-producing capacities of stem and leaf are, in the aggregate, only about 10 per cent of the capacity of the root system of the individual tobacco plant.[4] Obviously, the essential character of the observed rate dependency is open for some interesting investigation.

We know that nicotine behaves as a terminal metabolite in the plant inasmuch as it has a very low rate of turnover. Furthermore, the tobacco shoot grafted to tomato roots can grow as well without the customary contribution of nicotine from the tobacco root system as it does with it.[5] Attempts to demonstrate specific physiological effects of nicotine upon plant cells and tissues have never resulted in anything really clear-cut to compare with known effects upon insect and mammalian systems. Therefore, if the significance of the correlation with growth rate is to be explained, it would appear that the *process* of nicotine production, not the product, is the entity upon which attention should be fixed.

The Precursors of Nicotine

The excised root culture is an ideal system for investigating pathways of nicotine biosynthesis. We have prepared various isotope-labeled compounds, supplied them to the root cultures aseptically, and ascertained the extent and position of label accumulation in the alkaloid subsequently produced. The precursor of the pyridine ring has been identified as nicotinic acid.[6] The carboxyl group and the hydrogen atom in ring position 6 of the acid are lost in the transformation, but the remainder of the molecule appears to be transferred intact.[7]

Dewey *et al.*[8] and Leete[9] have shown that the diamino acid ornithine is the precursor of the pyrrolidine ring of nicotine. Ornithine is cyclized and combined with the pyridine ring precursor via a symmetrical intermediate.

The details of the reactions by which coupling of the two precursors is achieved by the cell are not yet known. They are of great potential importance, however, for reasons outlined in the next paragraphs.

The Rate-Limiting Step

A rather astonishing aspect of the rate dependency of nicotine yield upon root growth is the fact that the proportionality constant of the dependency cannot be altered by supplying excess quantities of either or both of the known precursors of the nicotine molecule to the root culture. Apparently, the isotopically-labeled molecules that contribute their labels to nicotine merely dilute a pool of precursors from which nicotine is produced at a rate independent of actual pool size.

It seems reasonable to conclude, therefore, that one of the chemical reactions lying between nicotinic acid and ornithine on the one hand and nicotine on the other is rate-limiting for nicotine biosynthesis. Even more important, it would seem that the same step is also rate-limiting for the over-all phenomenon of growth. The identity and nature of this step are not yet known, but such information will be awaited with great interest.

Nicotine Production in the Intact Tobacco Plant

The connection between nicotine production and growth in the aerial portions of the tobacco plant, the leaves and stems, is obscured by two factors.

The first is that the plant makes over 90 per cent of its nicotine in the root system[4] and then transports it principally to the leaves via the vascular system. The second is that the plant physiologist usually finds it much easier to measure plant growth in terms of increase in leaf or stem mass rather than root mass. Consequently little is known about the growth rates of the root systems of intact plants. Thus the kinetics of nicotine accumulation in the leaf do not reflect the kinetics of leaf growth. Indeed, leaf nicotine content may continue to increase after leaf growth has ceased. These facts are not fully comprehended by those who conduct applied research on tobacco crop production and by the grower. If they were understood, it seems entirely possible that methods for the control of nicotine content, especially in dry season crops, might be developed.

In a general sense, however, the mean level of nicotine accumulation in the leaf is controlled by genetic factors. Secondary modifications are induced by those environmental factors that affect the ratio of root to shoot mass and, perhaps, also by the relative intensity of inorganic nitrogen assimilation by the roots. The nature of genetic control is not understood fully as yet. It may be that the large differences that exist with respect to leaf nicotine content between varieties of cultivated tobaccos reflect corresponding differences in the proportionality constant relating nicotine yield to root growth. It is equally possible that inherited differences in the ratio of total root to total shoot mass may explain differences in leaf nicotine content. We have found that the excised roots of a Turkish cigarette type and a Pennsylvania cigar type of tobacco have the same proportionality constant between nicotine yield and growth despite the fact that the nicotine contents of their green leaves differ by a factor of two or more.

Metabolism of Nicotine

As stated earlier, nicotine turnover in the plant occurs at a low rate. If one examines the alkaloid fraction of the green leaf, one usually finds predominantly nicotine, varying quantities of nornicotine, anabasine, and anatabine, and trace amounts of some others of the pyridine class. It is not known into what compounds the small amounts of nicotine are metabolized, but the destruction is probably complete in the sense that the products of turnover are no longer recognizable as alkaloids.

Considerable interest attends the changes that occur during commercial processing, however. Most tobaccos are processed by an initial stage, called curing, which is essentially a more or less imperfectly controlled autolysis. Little nicotine is lost during this operation, but in some cigar tobacco types curing is following by another operation called fermentation. In this operation, considerable nicotine may be converted to partial degradation products including oxynicotine, 3-pyridylmethylketone, 3-pyridylpropylketone, *N*-methylnicotinamide, nicotinamide, nicotinic acid, myosmine, cotinine and 2,3′-dipyridyl.[10] Obviously, chemical attack has centered upon the pyrrolidine ring in most of these cases. The nature of the transformation products in the much smaller over-all changes that occur in cigarette tobacco aging have not been reported. Nevertheless, these pyridine derivatives, which are probably produced by leaf enzymes during the curing stage and by bacteria during the fermentation, may be expected to volatilize in the smoke along with nicotine and its combustion products, and they thus constitute at least a qualitatively important aspect of smoke chemistry.

Although we know little of the pathways of nicotine breakdown in the intact leaf, perhaps it is possible to infer something of the biochemical vulnerability of the nicotine molecule from the nature of the products listed above. In addition, there is a considerable volume of information about the pathways of nicotine breakdown by various bacteria. The general conclusion seems to be that, in most organisms, attack occurs first on the pyrrolidine ring and leads to greater or lesser attrition of that structure.[10] Although the stability of the pyridine ring seems to be much greater, there are known cases where the pyridine ring is attacked first, interestingly enough, in position 6 by hydroxylation, and the aromatic properties of the molecule are quickly destroyed.

Summary and Conclusions

The position of nicotine in plant biochemistry offers some interesting features.

Nicotine is produced by the tobacco plant from two extremely useful biochemical intermediates, ornithine of the arginine cycle and nicotinic acid of the pyridine dehydrogenase systems. It is not known what leads the plant to squander these valuable biochemical resources for the synthesis of a molecule that appears to play no further role in metabolism.

Looking at the process of synthesis rather than the product, we have discerned a close rate dependence between nicotine production and the growth of the plant root. Evidence is presented for the view that both root growth and nicotine synthesis are paced by the same rate-limiting step. Identification of this step can be of first-rate importance for further understanding of the still obscure biochemistry of growth as well as of the biochemistry of alkaloid formation.

Whatever the significance of the tie between nicotine production and growth, at present we can regard nicotine as an internal indicator of root growth, the actual amount present at any given instant representing a rather exact measure of the amount of growth that has occurred.

In answering the question posed at the beginning of this paper, it does not seem likely that plant biochemistry will be of much help in revealing the effects of nicotine on mammals. The facts that emerge are not comparisons, but rather contrasts. A useful purpose will have been served, however, if the fact that such contrasts exist can be incorporated into our general knowledge of the biochemistry of nicotine.

References

1. HARLAN, W. R. & J. M. MOSELEY. 1955. Tobacco. *In* Encyclopedia of Chemical Technology. **14:** 242–261. Interscience Encyclopedia, Inc. New York, N. Y.
2. WHITE, P. R. 1938. Cultivation of excised roots of dicotyledonous plants. Am. J. Botany. **25:** 348–356.
3. SOLT, M. L. 1957. Nicotine production and growth of excised tobacco root cultures. Plant Physiol. **32:** 480–484.
4. DAWSON, R. F. & M. L. SOLT. 1959. Estimated contributions of root and shoot to the nicotine content of the tobacco plant. Plant Physiol. **34:** 656–661.

5. Dawson, R. F. 1942. Accumulation of nicotine in reciprocal grafts of tomato and tobacco. Am. J. Botany. **29:** 66–71.
6. Dawson, R. F., D. R. Christman, R. C. Anderson, M. L. Solt, A. F. D'Adamo & U. Weiss. Biosynthesis of the pyridine ring of nicotine. J. Am. Chem. Soc. **78:** 2645.
7. Dawson, R. F., D. R. Christman, A. F. D'Adamo, M. L. Solt & A. P. Wolf. 1958. Pathway of nicotine biogenesis. Chem. & Ind. (London) : 100.
8. Dewey, L. J., R. U. Byerrum & C. D. Ball. 1955. The biosynthesis of the pyrrolidine ring of nicotine. Biochim. et Biophys. Acta. **18:** 141–142.
9. Leete, E. 1955. The biogenesis of nicotine. Chem. & Ind. (London) : 537.
10. Frankenburg, W. G., A. M. Gottscho & A. A. Vaitekunas. 1958. Biochemical conversions of some tobacco alkaloids. Tobacco. **146:** 20–24 (Tobacco Science. **2:** 9–13).

ABSORPTION OF NICOTINE FROM VARIOUS SITES

Janet Travell
Department of Pharmacology, Cornell University Medical College, New York, N. Y.

That hydrogen ion concentration is important in determining permeability of cell membranes to nicotine was established decades ago,[1-9] yet this principle is still slighted in current texts of pharmacology if, indeed, they mention it at all. For this reason, it is pertinent to review our early experiments and some unpublished data on the role of the *p*H in the absorption of alkaloids from various tissues.

Intact Skin

Nicotine poisoning after exposure of the skin to an insecticide spray was described by Faulkner[3] and others[10,11,12] from 1930 to 1933. Faulkner's case was that of a florist who sat on a bench wet with a 40 per cent aqueous spray solution of nicotine and 15 min. later, became critically ill with nicotine poisoning. The florist was hospitalized for 4 days and recovered. On discharge, he dressed in his clothes, which had been tied in a paper bag and stored when he entered the hospital; the seat of the trousers was still damp. One hour later, he had a second attack of nicotine poisoning.

Following these events, Faulkner[3] showed in 5 cats that nicotine in 40 per cent aqueous solution was absorbed through the intact skin; doses of about 280 to 1500 mg./kg. caused death in 21 to 98 min. On the other hand, in 3 cats a dose of about 1100 mg./kg. of nicotine sulfate, likewise in 40 per cent aqueous solution, had no effects. The sites of application were covered by an airtight cup.

As a result of this report, I demonstrated annually to pharmacology classes the striking difference in the percutaneous absorption of nicotine applied in acid and alkaline solutions. Thus, my series of 21 paired experiments extended over 21 years.

Method. Two cats of essentially the same weight were prepared the day before the experiment by clipping the fur from about a 5 × 6 cm. area of skin over the groin; care was taken not to abrade the skin. One cat received nicotine as the base and the other, an identical amount of nicotine (200 mg.) as the sulfate; the concentration of nicotine in each instance was 40 per cent with respect to the base. Weights of the paired cats ranged from about 2 to 3 kg., and the percutaneous dose of nicotine was thus about 66 to 100 mg./kg. Solutions were prepared by rapidly weighing the fluid nicotine oil and diluting it to volume with either distilled water or a solution of sulfuric acid to provide a slight excess of acid above the theoretical neutralization equivalent. The *p*H of the solutions was not determined but, on testing with litmus paper, one was found to be alkaline and the other acid. For application of nicotine and nicotine sulfate, animals were restrained on their backs, and 0.5 cc. of each solution was introduced from a tuberculin syringe onto the hollow of the groin and rubbed into the skin by the rubber-gloved finger for one minute. Some loss in the fur and on the rubber glove was unavoidable. Cats were released within a few minutes, and nicotine effects were often already evident in the case of the free base. Subsequently the animals were watched to insure that they did not lick the site of application. The site was not covered, but the method largely precluded significant ingestion or inhalation of nicotine.

Results. Results are summarized in TABLE 1. When nicotine was applied as the free base, signs of poisoning appeared in from 1 to 4 min., including ear flicking, licking, and swallowing, salivation, and increased rate of respiration. Vomiting occurred in all animals in 3.5 to 19 min. (average 9.8 min.). Death occurred in 17 of the 21 animals (81 per cent) in 21 to 195 min. (average 67 min.). The 4 surviving animals vomited in 10, 11, 15, and 19 min., respectively; all 4 were profoundly depressed, and as late as 4 hours after nicotine application one was described as "moribund."

In the case of nicotine sulfate, no signs of nicotine absorption were observed in 9 of the 21 cats (43 per cent). In the remaining 12 animals, the onset of early signs of poisoning (ear flicking, licking, swallowing, and panting) was appreciably delayed as compared with the group that received nicotine base, and these effects lasted for periods up to 90 min. in the absence of signs of more advanced toxicity (vomiting, general depression, and convulsions). Vomiting occurred in only 4 animals (19 per cent) in 15 to 69 min. after drug application. None of the animals died.

TABLE 1

PERCUTANEOUS EFFECTS OF NICOTINE (200 MG.) APPLIED IN ACID AND ALKALINE 40 PER CENT AQUEOUS SOLUTIONS TO THE INTACT SKIN OF THE CAT*

Nicotine solution	Total cats (no.)	Incidence of		
		Nausea (%)	Vomiting (%)	Death (%)
Alkaline	21	100	100	81
Acid	21	52	19	0

* Cats of equal weight were paired for acid and alkaline applications.

Discussion. Although the manner of applying the drug, the pH and the dose were subject to a number of variables in these experiments, they nevertheless confirm Faulkner's observation that nicotine is absorbed rapidly through the intact skin from a 40 per cent aqueous solution of its base and very slowly from a 40 per cent aqueous solution of its sulfate. Under the conditions of our experiments, the percutaneous absorption of nicotine, applied as the sulfate, keeps pace approximately with its elimination, which is of the order of 1 mg./kg./hour.

This value for speed of elimination of nicotine is based on the observation[7] that nicotine base can be injected subcutaneously in cats at the rate of 1 mg./kg./hour for several days without causing death provided the single dose does not exceed 6 mg./kg. given at intervals of 6 hours, but when the rate of injection is increased to 2 mg./kg./hour, death ensues after many hours because of cumulation.

Subcutaneous Tissue

The literature presents conflicting statements on the influence of the *p*H on nicotine absorption from subcutaneous tissues. Moore and Row[1] reported nicotine base to be 5 times as active as its hydrochloride when injected subcutaneously in frogs. Haag and Neal[13] found that the toxicity of nicotine injected subcutaneously in mice was only slightly greater than that of nicotine sulfate or salicylate. The *p*H of their solutions was not stated, but we determined that a 1 per cent (w/v) aqueous solution of nicotine as the base has a *p*H of 10.1, and as the sulfate, a *p*H of 4.3. One explanation of the discrepancy in the results is that the mechanism for bringing the *p*H of the subcutaneously injected solution to that of plasma operates more efficiently in the mouse than in the frog. To prolong this *p*H gradient I injected nicotine in buffered solutions at an acid and alkaline *p*H.[6]

Method. In 3 groups of 6 cats nicotine was injected subcutaneously in doses of 6, 7.5, and 9 mg./kg. in an aqueous solution buffered at *p*H 9.0. Similarly, in 3 groups of 6 cats nicotine was injected in doses of 9, 12, and 15 mg./kg. in a solution buffered at *p*H 4.8. Buffers were phthalates, phosphates, and sodium bicarbonate. The *p*H was determined electrometrically (glass electrode). The volume injected was constant, namely, 5 cc./kg. The site of the subcutaneous injection was the inguinal fold as it was lifted from the underlying musculature. The fluid disappeared rapidly from the site of injection.

In another series of experiments[7] nicotine was prepared as a 1 per cent aqueous solution without buffers (*p*H 10.1), and doses of 6, 7, and 8 mg./kg. (9 cats) and 9 mg./kg. (3 cats) were injected subcutaneously in the inguinal fold in the more usual smaller volumes of 0.6 to 0.9 cc./kg.

Results. When nicotine was injected subcutaneously (FIGURE 1) at the buffered *p*H of 9.0, a dose of 9 mg./kg. was fatal to 83 per cent of the animals in 25 to 36 min. (average 32 min.), and the smaller doses did not cause death. On the other hand, at the buffered *p*H of 4.8 the 9 mg./kg. dose was survived, and a dose of 15 mg./kg. produced effects similar to those of the 9 mg./kg. alkali buffered dose, that is, death in 67 per cent of the animals in 24 to 50 min. (average 32 min.). The intermediate dose of 12 mg./kg., buffered at *p*H 4.8, proved fatal in 33 per cent of the animals after a somewhat longer period of time, namely, 56 and 200 min.

In the cats that received subcutaneous nicotine in a small volume of solution at *p*H 10.1, the effects were essentially the same as for nicotine injected at the buffered *p*H of 9.0 in 5 cc./kg. of fluid; thus, doses of 6 to 8 mg./kg. did not cause death in any of the 9 cats, whereas 9 mg./kg. was fatal to all of 3 animals in 15, 23, and 38 min.

Discussion. These experiments indicate that the hydrogen ion concentration of a buffered solution within a certain *p*H range may modify the speed of absorption of nicotine from subcutaneous tissues. The toxicity of nicotine decreased about 60 per cent when the *p*H of the buffered solution was reduced from 9.0 to 4.8 and variables in the injection technique, such as the volume and site of injection, were standardized.

Urinary Bladder

Following my observation[4,5] that alkaloids are well absorbed through the gastric mucosa from alkaline solutions, it occurred to me that the urinary bladder mucosa might also be permeable to these weak bases under similar conditions.[6] At that time no studies on the role of the *p*H in the absorption of alkaloids from the urinary bladder were known to me.

Method. Under light ether anesthesia, the ureters were ligated in 10 cats and a cannula was inserted into the urethra. The bladder was washed with physiological saline, and nicotine in doses of 10 or 20 mg./kg. was instilled in a buffered solution. As in the experiments on subcutaneous absorption of nicotine, phthalates, phosphates, and sodium bicarbonate were used as buffers, the volume of fluid was 5 cc./kg., and the *p*H of solution injected into, or withdrawn from, the bladder 4 hours later or at death was measured electrometrically (glass electrode).

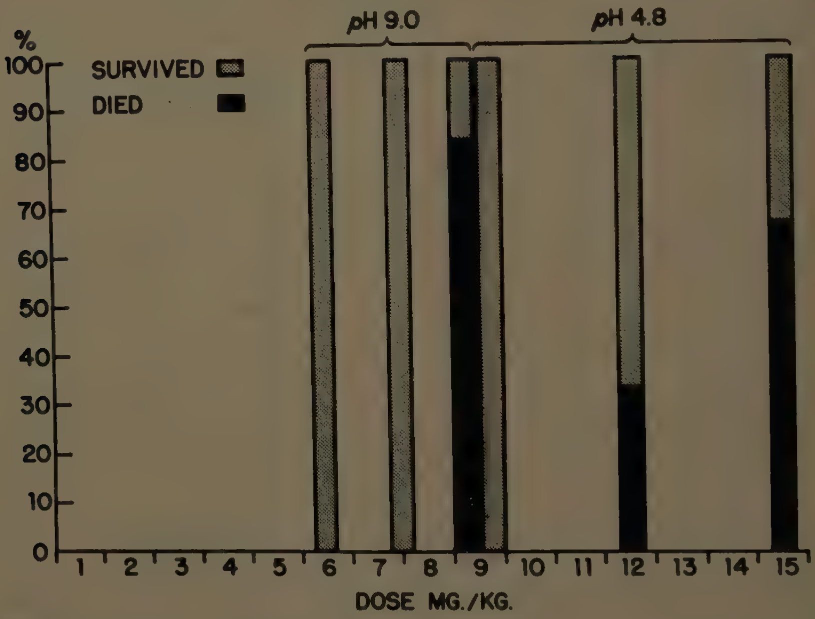

FIGURE 1. Influence of *p*H on toxicity of nicotine injected subcutaneously in aqueous buffered solution (six cats in each group).

Results. The results are shown in TABLE 2. At *p*H levels of 4.6 to 5.8, doses of 10 and 20 mg./kg. of nicotine produced no effects (cats 1 and 2). The same doses in the *p*H range of 7.0 to 7.9 caused fairly marked poisoning (cats 3, 4, 5, and 6). At *p*H levels of 8.0 to 9.0, the 10 mg./kg. dose of nicotine caused very marked poisoning (cats 7 and 8), and 20 mg./kg. was fatal (cats 9 and 10).

The rapidity of absorption of nicotine through the urinary bladder is shown by advancement of the nictitating membrane in 5 or 6 min. after instillation of the drug at an alkaline *p*H. The duration of nictitating membrane advancement in general paralleled the intensity of nicotine effects.

The *p*H was relatively stable during the period of the experiments, and the initial volume of fluid instilled was essentially the same as that recovered from the bladder terminally. There was no irritation of the bladder mucosa on gross postmortem examination.

Discussion. Sollman's text[14] states that "nicotine is absorbed rapidly from mucous membranes . . .; more slowly from hypodermic injections." Our experiments suggest that, when injected at a comparable *p*H and in the same concentration, the absorption of nicotine appears to be slightly greater from subcutaneous tissue spaces than from the mucosa of the urinary bladder. However, since the volume of fluid in the bladder was unchanged, as nicotine was absorbed its concentration necessarily decreased. This would result in a diminishing rate of absorption, and if the initial concentration were not high enough to cause death, nicotine effects might diminish while a significant amount of nicotine remained in the bladder. The rapid absorption of fluid given by the subcutaneous route would tend to maintain the original concentration of nicotine in this tissue and insure virtually complete absorption of the alkaloid. In view of these considerations, the absorbing capacity of subcutaneous tissues and of the urinary mucosa for nicotine in buffered solution are probably of the same order.

Crane[15] studied the influence of *p*H on the toxicity of various alkaloids (caffeine, atropine, quinine, strychnine, *d*-coniine and piperidine) for Paramecia,

TABLE 2

INFLUENCE OF *p*H ON ABSORPTION OF NICOTINE FROM LIGATED URINARY BLADDER*

Cat no.	*p*H of solution		Nicotine injected into bladder‡ (mg./kg.)	Nictitating membrane advancement		Degree of nicotine poisoning	Calculated concentration undissociated nicotine base injected
	Injected	Recovered†		Onset (min.)	Duration (min.)		
1	4.6	4.7	10	—	0	none	1:1,250,000
2	5.4	5.8	20	—	0	none	1:83,000
3	7.2	7.0	10	24	19	moderate	1:3,300
4	7.9	7.4	10	21	50	marked	1:1,000
5	7.0	6.8	20	30	54	marked	1:2,500
6	7.9	7.0	20	20	90	marked	1:520
7	8.6	8.1	10	5	180	very marked	1:630
8	8.7	8.1	10	10	148	very marked	1:590
9	9.0	9.0	20	5	—	fatal 17 min.	1:270
10	9.0	8.9	20	6	—	fatal 40 min.	1:270

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† When sacrificed 4 hr. after injection or at death.

‡ Injected in a volume of 5 cc./kg.

and concluded that cell membranes are readily permeable to the lipid-soluble undissociated alkaloidal bases, but not to their ions. The free base is apparently responsible for toxicity. I applied this hypothesis to my data (TABLE 2) and found that the degree of nicotine poisoning did indeed parallel the calculated concentration of undissociated nicotine base injected into the bladder lumen. Drug effects indicated that nicotine absorption exceeded elimination when the concentration of free base was 0.03 to 0.1 per cent; very marked poisoning occurred at concentrations of about 0.15 to 0.2 per cent, and death resulted at a concentration of 0.37 per cent. Calculations were based on the formula previously derived[5,6] and taking the dissociation constant of nicotine[16] as 9×10^{-7}:*

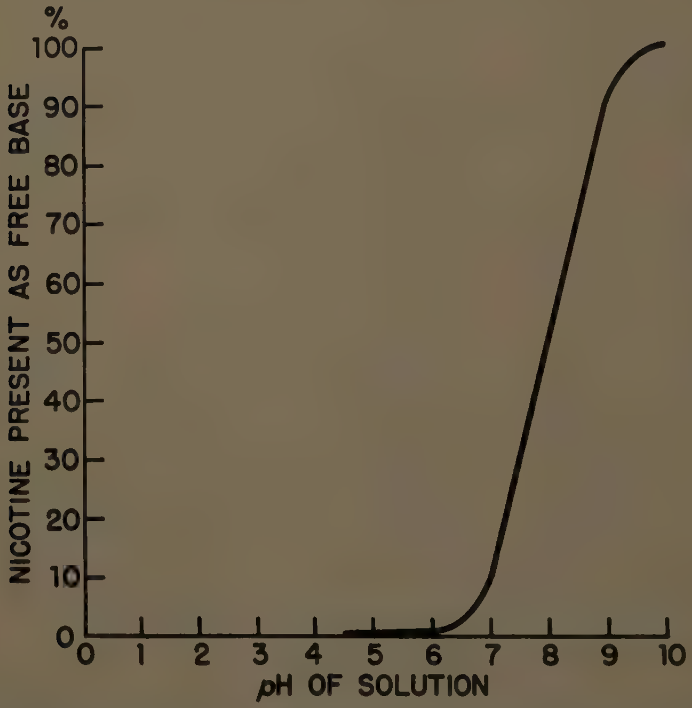

FIGURE 2. Dissociation curve of nicotine in aqueous solution, expressed as per cent of undissociated (free) base.

$$\frac{[\text{Undissociated nicotine}]}{[\text{Nicotine ions}]} = \frac{(1 \times 10^{-14})}{(9 \times 10^{-7})\ (1 \times 10^{-pH})}.$$

The proportion of the total nicotine present as free base in aqueous solution at different hydrogen ion concentrations is shown in FIGURE 2. Steepening of the slope of the dissociation curve lies within the physiological range of *p*H change for normal tissues. Thus at *p*H 5.0, only 0.2 per cent of the total nicotine is present as free base; at *p*H 6.0, 1 per cent is free base, a 5-fold increase; at *p*H 6.5, 2 per cent is free base, a 10-fold increase; at *p*H 7.0, 10 per cent is free base, a 50-fold increase, and at *p*H 8.0, 52 per cent is free base, more than a 250-fold increase from *p*H 5.0. This means that if alkaloids permeate lipid membranes in the form of lipid-soluble undissociated base, the degree of ionization in solution largely governs their rates of absorption.

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* The K_b of nicotine was also determined by Weatherby[8] as 8.07.

Since appreciable amounts of absorbed nicotine are excreted unchanged in the urine, the urinary *p*H should determine in part the total nicotine eliminated by this route. At an alkaline *p*H, reabsorption of nicotine from the lumen of the bladder would take place with further metabolic degradation of the molecule during each excretion-reabsorption cycle. It was in fact determined[17,18] that one tobacco smoker (inhaler) who retained 115 mg. of nicotine from the smoke of 40 cigarettes in 24 hours, eliminated 15.0 mg. of nicotine daily when the urine was acidified (*p*H 5.0 to 5.5) by oral administration of ammonium chloride, but only 2.7 mg. when the urine was alkalinized (*p*H 7.1 to 7.6) by administration of alkaline salts. The difference in the urinary yield was thus more than 5-fold, presumably due to reabsorption of nicotine.

My associates and I have shown[7] that on repeated subcutaneous injection of nicotine in the cat with ligated urethra, when the normally acid urine was alkalinized by addition of sodium bicarbonate, the animal was poisoned by the nicotine that had accumulated in its bladder and was reabsorbed in consequence of the alkalinization. Therefore we raised the question of whether the tobacco smoker who accumulated nicotine in acid urine within the bladder might develop signs of nicotine poisoning if the retained urine were suddenly alkalinized by an oral dose of sodium bicarbonate and the nicotine content absorbed. In the case of some heavy smokers (inhalers), we found that fractions of the night sample of acid urine caused marked nicotine effects when injected intravenously in cats, and even caused death. We calculated that these urines contained sufficient nicotine to cause poisoning in man, if we assume that the alkaloid is similarly absorbed from the human bladder when the urine is alkaline. That the absorption of nicotine from the urinary bladder at an alkaline *p*H is not peculiar to the cat is shown by similar results in the dog.[19]

Gastrointestinal Tract

More than twenty years ago a medical student asked, "Is a tablet of morphine really more effective when held under the tongue than when it is swallowed?" "Yes, indeed," I replied, and then began to seek the experimental basis for my answer.

A search of the literature yielded conflicting statements concerning the absorption of alkaloids from the stomach, and no studies on buccal absorption were available. The most obvious difference in the milieu of the mouth and of the stomach was the hydrogen ion concentration, and I started to investigate[4,5] the role of the *p*H in the gastrointestinal absorption of alkaloids. Important contributions to this subject have since followed.[20-24]

Method. The alkaloids studied were nicotine, strychnine, cocaine, atropine, and physostigmine.

Preliminary experiments on gastric absorption in rabbits proved unsatisfactory because of the long period of fasting required for the stomach to empty and because of difficulty in neutralizing gastric acidity. Therefore, in 55 fasted cats (and 3 dogs) under ether anesthesia and procaine infiltration of the abdominal wall, the stomach was ligated at cardia and pylorus so as to exclude the large blood vessels along the curvatures of the stomach. After withdrawal of air, the alkaloidal solution was injected directly into the lumen in the antral region. The total volume was always 15 cc./kg. An alkaline *p*H was obtained by diluting 5 cc. of an aqueous solution of the alkaloidal salt with 10 cc. of 1 per cent sodium bicarbonate to make the dose of the latter 100 mg./kg. and the initial *p*H of the injected solution 8.0 to 8.6. Mixing of the warm solutions was done just before injection, and a sometimes supersaturated clear solution of the relatively water-insoluble alkaloidal base was thus prepared and immediately injected. An acid *p*H was obtained by similarly diluting 5 cc. of the alkaloidal salt solution with 10 cc. of either 0.1 *N* hydrochloric acid to make the *p*H 1.2, or of physiological saline solution to make the *p*H 5.7. If death occurred, the volume of residual fluid in the stomach was measured, the final *p*H determined, and a postmortem examination performed. Similar examinations were carried out in those animals that survived and were sacrificed in 24 hours. In 2 surviving cats, 24 hours after the alkaloid had been given in acid solution, the gastric fluid was withdrawn, alkalinized with sodium bicarbonate, and reintroduced into the stomach. In a series of 8 cats samples of gastric fluid were withdrawn at intervals for measurement of intermediate *p*H changes. The *p*H was determined electrometrically (glass electrode) or colorimetrically; readings by the two methods usually checked within 0.1 *p*H and did not differ by more than 0.3 *p*H.

The influence of local irritation on the absorption of an alkaloid at an acid *p*H from the ligated stomach was studied in 15 cats by means of ethyl alcohol and in 4 cats by means of salicylic acid. To 5 cc. of the solution of the alkaloid in 0.15 *N* hydrochloric acid or physiological saline, 10 cc. of diluted U.S.P. ethyl alcohol was added, making its concentration 5, 10 or 25 per cent; thus, the dose of ethyl alcohol was 0.75, 1.5 or 3.8 cc./kg., respectively. Salicylic acid in a final concentration of 0.05 or 0.1 per cent was similarly employed.

In another series of 4 experiments, a loop of small intestine 50 cm. in length was ligated with the upper ligature at the pylorus. The alkaloid was injected into the lumen in 0.15 *N* hydrochloric acid at *p*H 1.2 in a volume of 15 cc./kg.

In 36 normal fasted cats, the alkaloid was given by stomach tube in acid or alkaline solution in a volume of 15 cc./kg., as in the ligated stomach and intestine, and the effects were observed.

Results: ligated stomach. Nicotine injected into the ligated stomach at *p*H 8.6 in a dose of 20 mg./kg. (at least twice the buffered subcutaneous fatal dose in cats) was fatal in 41 min., whereas 50 mg./kg. injected at *p*H 1.2 caused no effects, even though the intragastric *p*H 24 hours later had increased to 4.2 (TABLE 3).

Similarly, in the case of atropine, cocaine, and physostigmine (sulfate), 3 to 5 times the dose that proved fatal in alkaline solution was survived with only mild drug effects when given at an acid *p*H, and the final *p*H was not above

2.0 (TABLE 3). In the case of physostigmine sulfate in saline, 100 mg./kg. was not toxic and the final *p*H was about 2.0. However, 100 mg./kg. of physostigmine salicylate proved fatal whether given in saline or 0.15 *N* hydrochloric acid, and the terminal *p*H ranged from 2.8 to 5.8. One animal survived a 50 mg./kg. dose of physostigmine salicylate in saline and the final *p*H was 2.0. The concentration of salicylic acid in the injected solutions of physostigmine was calculated as about 0.1 and 0.2 per cent.

All doses of strychnine sulfate, even as small as 0.5 mg./kg., were fatal at an alkaline *p*H of 7.8 to 8.5 (TABLE 4). The larger the dose, the more rapid the onset of tetanus, and the shorter the interval from tetanus to death (FIGURE 3). On the other hand, doses of 5 to 50 mg./kg. given at *p*H 1.2 in 0.15 *N* hydrochloric acid, or at *p*H 5.7 in saline, were survived without strychnine effects when the final *p*H was 2.0 or less (TABLE 4). Thus in alkaline solution as little as 0.5 mg./kg. proved fatal in 109 min., but 100 times this dose at *p*H 2.0 was survived for 24 hours without drug effects.* At this time the stomach was still capable of absorbing strychnine rapidly, as shown by fatality in 2 experiments in which the intragastric fluid was withdrawn, alkalinized, and reintroduced into the stomach. When the final *p*H was as high as 3.4, 50 mg./kg. caused death in 106 min., approximating the rate of absorption of 0.5 mg./kg. at *p*H 8.0.

When the dose of strychnine sulfate was 5 mg./kg. and the intragastric *p*H

TABLE 3

EFFECT OF *p*H ON GASTRIC ABSORPTION OF ALKALOIDS*

Cat no.	Alkaloid	Dose† (mg./kg.)	*p*H of solution		Time to death (min.)
			Injected	At death	
1	Nicotine	20	8.6	7.8	41
2		50	1.2	4.2	Survived‡
3	Atropine sulfate	300	8.0	7.8	330
4		1000	1.2	2.0	Survived
5	Cocaine hydrochloride	200	7.7	7.5	167
6		1000	1.2	2.3	Survived
7	Physostigmine salicylate	15	8.2	7.9	39
8		50	5.7	1.5	Survived
9		100	1.2	3.0	35
10		100	5.7	5.0	49
11		100	5.7	2.8	113
12		100	5.7	5.8	165
13	Physostigmine sulfate	100	6.2	2.0	Survived

* Reproduced by permission of *Journal of Pharmacology and Experimental Therapeutics*.[5]
† Injected in a volume of 15 cc./kg.
‡ Surviving animals were normal when sacrificed 24 hours after injection.

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* Confirmatory results were obtained in 3 dogs.[5]

determined hourly,[5] strychnine effects were absent as long as the pH remained below 4.0; when it ranged from 5.0 to 6.0 poisoning developed in the course of 1 to 2 hours and death ensued. In these animals, disturbed hourly for aspiration of gastric fluid, the pH increased, possibly due to inhibition of acid secretion, in contrast to the low pH maintained in animals that were undisturbed (TABLE 4).

When the effects of 0.05 and 0.1 per cent concentrations of salicylic acid on strychnine absorption were studied for the ligated stomach (TABLE 5), it was found that strychnine sulfate (2 to 10 mg./kg.), given in saline with salicylic acid at pH 3.0 to 3.2, was uniformly fatal, in contrast to survival when this alkaloid (5 to 20 mg./kg.) was injected in saline alone at pH 5.7 to 5.8 (TABLE 4). In addition, the intragastric pH increased in the presence of salicylic acid, whereas it decreased in the surviving controls. On postmortem gross examination, those animals that had received strychnine with salicylic acid showed reddening and swelling of the gastric mucosa with marked mucus secretion, whereas surviving animals that had received alkaloids in either saline or hydrochloric acid without salicylic acid did not show such signs of local irritation

TABLE 4

EFFECT OF pH ON STRYCHNINE ABSORPTION FROM THE LIGATED STOMACH*

Cat no.	Strychnine sulfate† (mg./kg.)	pH of solution		Time to	
		Injected	At death	Tetanus (min.)	Death (min.)
		Alkaline			
1	0.5	8.5	8.0	68	109
2	1	8.5	7.8	45	48
3	2	8.5	8.2	27	50
4	5	8.5	8.3	14	24
5	10	8.5	8.2	13	24
6	20	8.5	8.5	11	13
		Acid			
7	5	1.2	1.6	0	Survived‡
8	5	5.8	1.8	0	Survived
9	5	5.8	2.0	0	Survived
10	10	5.8	1.6	0	Survived
11	20	1.2	1.8	0	Survived
12	20	5.8	1.9	0	Survived
13	50	1.2	2.8	?	7–18 hours§
14	50	5.7	3.4	77	106
15	50	5.7	2.0	0	Survived

* Reproduced by permission of *Journal of Pharmacology and Experimental Therapeutics*.[5]
† Injected in a volume of 15 cc./kg.
‡ Surviving animals were normal when sacrificed 24 hours after injection.
§ Died overnight; gross inflammation of stomach.

after comparable and even longer periods of ligation. Furthermore, the interval from tetanus to death in the presence of salicylic acid was only 2 or 3 min. and did not show a relationship to the size of the strychnine dose, in contrast to the lengthening of this interval with decreasing doses of strychnine given at an alkaline *p*H without salicylic acid (FIGURE 3).

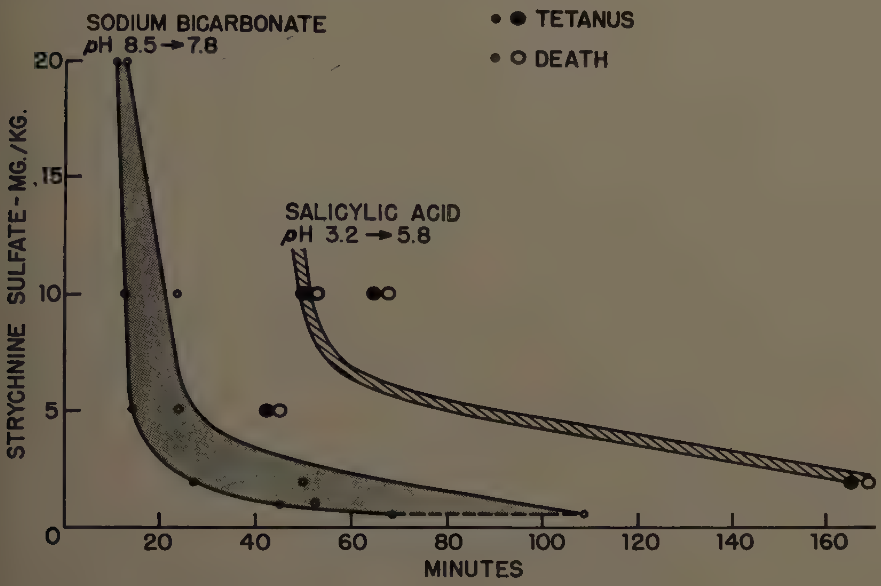

FIGURE 3. Dose-response curves of strychnine injected into the ligated stomach of cats in an aqueous solution rendered either alkaline (*p*H 8.5) by sodium bicarbonate or acid (*p*H 3.2) by salicylic acid.

TABLE 5
EFFECT OF SALICYLIC ACID ON ABSORPTION OF STRYCHNINE FROM THE LIGATED STOMACH

Cat no.	Strychnine sulfate* (mg./kg.)	Salicylic acid (%)	*p*H of solution		Time to	
			Injected	At death	Tetanus (min.)	Death (min.)
1	2	0.05	3.2	5.4	166	169
2	5	0.05	3.2	5.8	43	45
3	10	0.05	3.2	5.0	65	68
4	10	0.1	3.0	4.6	51	54

* Injected in a volume of 15 cc./kg.

When ethyl alcohol was added to strychnine injected at an acid *p*H into the ligated stomach, local irritation of the gastric mucosa was found to be an important factor in determining the rate of alkaloidal absorption (TABLE 6). In the presence of 25 per cent ethyl alcohol, strychnine sulfate (5 to 20 mg./kg.) injected in 0.15 *N* hydrochloric acid at *p*H 1.4 proved fatal in 5 of 6 experiments in from 39 to 180 min. This surprising result occurred in spite of the fact that such large doses of ethyl alcohol reduce the fatal dose of strychnine in the cat.[25] At death, however, marked reddening of the mucosa was present in all 5 animals, and the intragastric *p*H ranged from 4.2 to 5.9. The surviving animal in this group showed moderate gastric irritation and the final *p*H was 1.5. An explanation for the difference in the outcome may lie in the fact that in the latter experiment the volume of fluid recovered from the stomach was double that injected, whereas in the 5 fatalities the increase in volume averaged only 23 per cent (0 to 44 per cent). When strychnine in similar doses was given with 25 per cent ethyl alcohol in saline at *p*H 6.0, death resulted in all of 4 experiments in 21 to 35 min., and local irritation of the gastric mucosa was already evident. Gastric irritation by ethyl alcohol did not abolish the correlation of the tetanus-to-death interval with dosage (TABLE 6), as occurred with salicylic acid.

TABLE 6

INFLUENCE OF ETHYL ALCOHOL ON STRYCHNINE ABSORPTION FROM THE LIGATED STOMACH

Cat no.	Strychnine sulfate* (mg./kg.)	Ethyl alcohol (%)	*p*H of solution		Time to		Irritation of mucosa
			Injected	At death	Tetanus (min.)	Death (min.)	
1	5	25	1.4	1.5	none	survived	moderate
2	5			5.9	75	180	marked
3	10			4.2	29	91	marked
4	10			4.6	23	84	marked
5	20			4.5	59	113	marked
6	20			4.5	33	39	marked
7	5	25	6.0	8.0	13	21	moderate
8	10			5.8	20	26	slight
9	20			6.3	33	35	moderate
10	20			6.4	25	43	moderate
11	10	10	6.2	1.8	85	survived	none
12	10			3.0	35	survived	none
13	10			4.0	38	74	none
14	10	5	6.2	1.4	none	survived	none
15	10			1.8	none	survived	none

* Injected in a volume of 15 cc./kg.

Lower concentrations of ethyl alcohol (TABLE 6) did not cause gross irritation of the stomach, and 4 of 5 animals survived that received strychnine (10 mg./kg.) in saline and 5 or 10 per cent ethyl alcohol, even though the *p*H of the solution injected was 6.2. In the 4 surviving animals, the final *p*H ranged from 1.4 to 3.0 and, in the fatal result, it was 4.0. Volume of fluid recovered from the stomach showed no change or decreased slightly for the lower concentrations of ethyl alcohol, as was shown for administration in saline alone.

Results: ligated small intestine. When injected in 0.15 *N* hydrochloric acid at *p*H 1.2 in a volume of 15 cc./kg., strychnine was rapidly absorbed from the ligated small intestine (TABLE 7). Thus doses of 1, 2, and 5 mg./kg. proved rapidly fatal to 3 cats in 30 to 34 min.; in this time the *p*H had risen from 1.2 to a terminal *p*H of 4.6, 4.7, and 5.9 respectively. In one animal, 0.5 mg./kg. of strychnine sulfate caused tetanus in 73 min. with subsequent recovery, and the *p*H about 4 hours after injection was 7.3.

Results: oral administration. When strychnine sulfate was administered by stomach tube to the normal cat in alkaline, slightly acid, or strongly acid solution (TABLE 8), rate of absorption was influenced to some extent by the *p*H. Thus at *p*H 8.0, strychnine sulfate in doses of 0.4 to 1.0 mg./kg. was fatal in 75 per cent of 12 animals, and tetanus developed in 100 per cent in an average of 31 min. When strychnine was given in the same dosage to the same number of cats, but in saline at *p*H 5.7, only 33 per cent died and tetanus developed in 75 per cent in an average of 34 min. When the drug was similarly given in hydrochloric acid at *p*H 1.2 to 12 animals, mortality rate and incidence of tetanus were essentially the same as for administration in saline, but the interval to tetanus was doubled (69 min.).

TABLE 7
ABSORPTION OF STRYCHNINE FROM A LIGATED LOOP OF SMALL INTESTINE

Cat no.	Strychnine sulfate* (mg./kg.)	*p*H of solution		Time to	
		Injected	At death	Tetanus (min.)	Death (min.)
1	0.5	1.2	7.3	73	S†
2	1.0	1.2	5.9	32	34
3	2.0	1.2	4.7	23	30
4	5.0	1.2	4.6	27	30

* Injected in a volume of 15 cc./kg.
† Marked recovery from strychnine effects when sacrificed 255 min. after injection.

TABLE 8
INFLUENCE OF *p*H ON TOXICITY OF STRYCHNINE ADMINISTERED ORALLY IN 36 NORMAL CATS

Reaction of solution	Strychnine sulfate* (mg./kg.)	Total cats (no.)	Tetanus		Time to tetanus (min.)†	Died	
			(no.)	(%)		(no.)	(%)
Alkaline ($NaHCO_3$)	0.4	4	4	100	31 (17–55)	2	75
	0.6	4	4			3	
	0.8–1.0	4	4			3	
Slightly acid (NaCl)	0.4	4	1	75	34 (20–49)	0	33
	0.6	4	3			0	
	0.8–1.0	4	4			4	
Strongly acid (HCl)	0.4	4	1	58	69 (38–109)	1	42
	0.6	4	2			1	
	0.8	4	4			3	

* Volume injected was 15 cc./kg.
† Figures in parentheses indicate range.

Discussion. The *p*H dependence of gastric absorption applies not only to nicotine, which has unusual physical properties, but is a general alkaloidal phenomenon that I have demonstrated in these toxicity studies for various alkaloids in the cat, as well as in the dog.[5] Schanker *et al.*[20] established this principle in the rat by direct quantitative determination; for example, in 1 hour the rat stomach absorbed none of the quinine in 0.1 *M* hydrochloric acid, 7 per cent in saline, and 18 per cent in sodium bicarbonate at *p*H 8. For acidic drugs the reverse relationship exists.[20] Analogous to the urinary bladder, a lipid barrier was postulated[20] between plasma and gastric juice, which permits passive transfer of drugs in their lipid-soluble un-ionized form, but not in their lipid-insoluble ionized form.

The degree of ionization of an organic electrolyte in solution is determined primarily by the ionization constant of the compound and the *p*H of the solution. Other factors modifying speed of absorption include the concentration and dose of the drug, and the size, blood flow, and health of the absorbing surface.

Like the phospholipid membrane system of Weatherby,[9] the undamaged gastric mucous membrane of the cat was virtually impermeable to nicotine at a *p*H not above 4.2 for as long as 24 hours. Strychnine, cocaine, atropine, and physostigmine were also poorly absorbed from the stomach when the intragastric *p*H was less than 4.0, and rapidly absorbed when it was 5.0 or higher. When the gastric mucosa was altered by an irritant concentration of salicylic acid (0.05 to 0.1 per cent), or of ethyl alcohol (25 per cent), strychnine* was rapidly fatal when given at an acid *p*H of 3.2 or 1.4, respectively. Vasodilatation, a rapid rise in *p*H due to outpouring of mucus, and cellular damage may have been factors in the outcome. The modifying influence of local blood flow on alkaloidal absorption has been shown for nicotine injected intraperitoneally in the mouse;[26] thus, 1:24,000 epinephrine given simultaneously by the same route reduced the fatality of nicotine acetate from about 40 to 23 per cent, and 0.056 *M* formaldehyde, a local irritant, increased it to 63 per cent. Likewise, the retardation of procaine absorption by added epinephrine is well known.

In the case of salicylic acid, actual destruction of the epithelial barrier probably played a role in the acceleration of strychnine absorption from the stomach, since death followed tetanus almost at once regardless of the dose (FIGURE 3); with 25 per cent ethyl alcohol this interval was longer for the smaller doses of strychnine, as when gross irritation of the gastric mucosa was not present. Breakdown of the gastric mucosal barrier by salicylic acid would explain the prompt absorption of physostigmine, given as the salicylate in strongly acid solution, but not when given as the sulfate. It might also explain the discrepancy noted by Schanker *et al.* in the unexpectedly high absorption of salicylic acid itself at an alkaline *p*H by the rat stomach[20] and small intestine.[22,23] These investigators note[22] that at *p*H 6.6, the ratio of un-ionized to ionized salicylic acid is about 1 to 4000, and "this seems too small a proportion of un-ionized drug to explain the rapid absorption of the compound."

From the ligated small intestine in the cat, strychnine was rapidly absorbed when injected in hydrochloric acid at *p*H 1.2. Coincident with absorption, there was rapid neutralization of acid and *p*H rise to levels at which the alkaloid was readily absorbed from the stomach. The *p*H dependence of intestinal absorption was clearly demonstrated in the rat small intestine[22,23] and colon[24] by Schanker *et al.* and Hogben *et al.*; they showed that the absorption of basic drugs was increased severalfold when the *p*H of the intestinal contents was increased from 4 to 8, and conversely for acidic drugs.

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* The ionization constant of strychnine is in the same range as that of nicotine, namely, 8.5×10^{-7} at 18° C.[5]

On oral administration of strychnine to the intact cat, addition of sodium bicarbonate significantly increased the toxicity of the alkaloid, probably because of accelerated absorption from the stomach. When the effects of similar strychnine administration in saline and in hydrochloric acid were compared, the ultimate toxicity of strychnine was found to be essentially the same for the two groups. However, the time to the onset of poisoning was approximately doubled by the administration of hydrochloric acid; the delay probably represents the time needed to neutralize the excess acid in the small intestine.

These observations suggest certain rules for the treatment of alkaloidal poisons taken by mouth: (1) never give sodium bicarbonate; (2) administer an acid of physiological strength, such as vinegar; (3) never give alcohol.

Mouth

No studies were carried out on the role of *p*H in the buccal absorption of alkaloids, but investigations in this area should prove fruitful in relation to the oral absorption of nicotine from tobacco smoke, especially in those individuals who do not inhale.

Buccal tablets are available for quick onset of effects of some alkaloids, for example, of ergotamine and Isuprel. In this connection the variations in the *p*H of the mouth become of some importance. Brawley[27,28] reviewed the literature on this subject and measured variations in the *p*H of the "resting saliva" with age, sex, and diurnal changes, and found the range to be from 5.6 to 7.8; other investigators extended the range from 5.0 to 8.0. More recently, Sullivan *et al.*[29] determined the *p*H for various regions of the mouth using a microglass electrode, and report the range to be from 5.5 to 7.3.

We made some observations on the *p*H of the mouth at the tip of the tongue in relation to cigarette smoking and eating; *p*H was determined by nitrazine paper. An illustrative experiment on a habitual smoker is shown in FIGURE 4. In all the subjects tested either smoking or eating, and in some instances even talking about food, caused an immediate rise in the *p*H of this region of the mouth, probably due to an increased flow of saliva.

Summary and Conclusions

Influence of *p*H on nicotine absorption from various sites was measured indirectly by speed of onset, intensity, and duration of pharmacological effects in the cat. Sites studied in relation to a physiologic range of *p*H change were the intact skin, subcutaneous tissue, ligated urinary bladder, and stomach. The role of *p*H in the absorption of some other alkaloids (strychnine, atropine, cocaine, and physostigmine) was investigated for the ligated stomach and small intestine and also after oral administration in the intact cat.

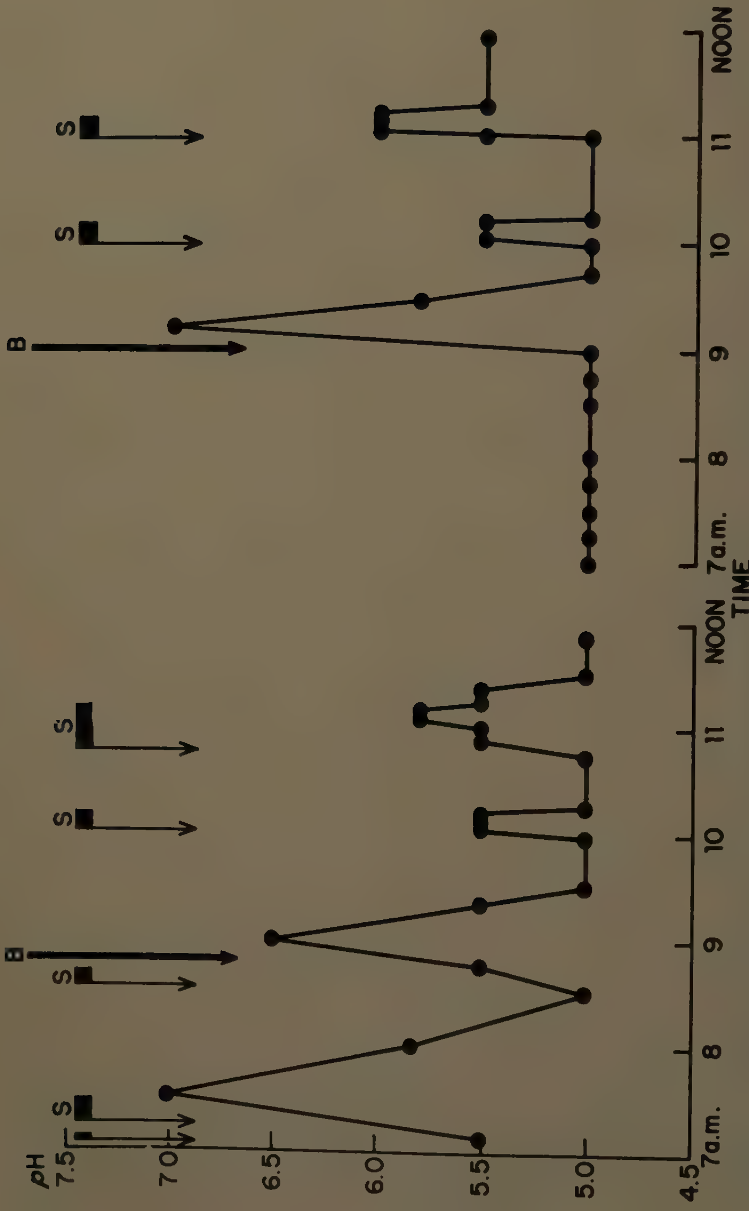

Figure 4. Effect of smoking (S) 1 to 4 cigarettes (Turkish and domestic blend without filter) and of eating breakfast (B) on *p*H of the mouth in a habitual smoker (inhaler).

The data indicate that:

(1) Through the intact skin nicotine is absorbed rapidly from a 40 per cent aqueous solution of its base, and very slowly from a 40 per cent aqueous solution in the form of the sulfate.

(2) For subcutaneous tissue the *p*H modifies the speed of absorption of nicotine injected in buffered solution.

(3) From the urinary bladder nicotine is rapidly absorbed at *p*H levels of 7.0 or higher, but slowly when the *p*H is below 6.0.

(4) The degree of nicotine poisoning parallels the calculated concentration of undissociated nicotine base in aqueous solution within the urinary bladder.

(5) The absorbing capacity of subcutaneous tissue and of the urinary mucosa for nicotine in buffered solution and identical concentrations are of the same order.

(6) From the stomach nicotine is rapidly absorbed at an alkaline *p*H, but practically not at all at a *p*H of about 4.0.

(7) The *p*H dependence of gastric absorption applies not only to nicotine but also to the other alkaloids studied: namely, cocaine, atropine, physostigmine, and strychnine.

(8) Salicylic acid and ethyl alcohol in irritant concentrations vitiate the limiting influence of the normal gastric acidity on alkaloidal absorption from the stomach by causing vasodilatation, mucus secretion, a rise in the *p*H, and breakdown of the mucosa:plasma barrier.

(9) Absorption of an orally administered alkaloid (strychnine) in the intact animal may be materially delayed, and the intensity of poisoning reduced, by simultaneous administration of 0.1 *N* hydrochloric acid, whereas sodium bicarbonate increases its toxicity.

References

1. Moore, B. & R. Row. 1897. A comparison of the physiological actions and chemical constitution of piperidine, coniine and nicotine. J. Physiol. **22:** 273.
2. Ellisor, L. O. & C. H. Richardson. 1938. Penetration of nicotine into the goldfish from solutions of various hydrogen ion concentrations. J. Cellular Comp. Physiol. **11:** 377.
3. Faulkner, J. M. 1933. Nicotine poisoning by absorption through the skin. J. Am. Med. Assoc. **100:** 1664.
4. Travell, J. 1938. The absorption of alkaloids from the stomach. J. Pharmacol. Exptl. Therap. **63:** 37.
5. Travell, J. 1940. The influence of the hydrogen ion concentration on the absorption of alkaloids from the stomach. J. Pharmacol. Exptl. Therap. **69:** 21.
6. Travell, J. 1940. Influence of *p*H on absorption of nicotine from urinary bladder and subcutaneous tissues. Proc. Soc. Exptl. Biol. Med. **45:** 552.
7. Travell, J., O. Bodansky & H. Gold. 1940. Nicotine excretion and the role of its reabsorption from the urinary bladder as a source of nicotine poisoning in tobacco smokers. J. Pharmacol. Exptl. Therap. **69:** 307.
8. Weatherby, J. H. 1940. Influence of H ion on depressant effect of nicotine solutions on ciliated epithelium. Proc. Soc. Exptl. Biol. Med. **45:** 284.
9. Weatherby, J. H. 1949. Permeability of the artificial phospholipid membrane. J. Cellular Comp. Physiol. **33:** 333.
10. Wilson, D. J. B. 1930. Nicotine poisoning by absorption through the skin. Brit. Med. J. **2:** 601.
11. Fretwurst, F. & A. Hertz. 1932. Akute nikotinvergiftung. Z. klin. Med. **122:** 641.
12. Lockhart, L. P. 1933. Nicotine poisoning. Brit. Med. J. **1:** 246.
13. Haag, H. B. & R. C. Neal. 1940. Comparative toxicity of nicotine and some of its salts. J. Pharmacol. Exptl. Therap. **69:** 289.

14. Sollmann, T. 1958. A Manual of Pharmacology. : 455. 8th ed. Saunders. Philadelphia, Pa.
15. Crane, M. M. 1921. The effect of hydrogen ion concentration on the toxicity of alkaloids for *Paramoecium*. J. Pharmacol. Exptl. Therap. **18:** 319.
16. Craig, L. C. & R. M. Hixon. 1931. Electron sharing ability of organic radicals. Nitrogen heterocyclics. J. Am. Chem. Soc. **53:** 4367.
17. Haag, H. B. & P. S. Larson. 1942. Urinary excretion of nicotine by smokers. Federation Proc. **1:** 5.
18. Haag, H. B. & P. S. Larson. 1942. Studies on the fate of nicotine in the body. I. The effect of *p*H on the urinary excretion of nicotine by tobacco smokers. J. Pharmacol. Exptl. Therap. **76:** 235.
19. Borzelleca, J. F. 1960. Nicotine absorption from the urinary bladder of the dog. Federation Proc. **19:** 391.
20. Schanker, L. S., P. A. Shore, B. B. Brodie & C. A. M. Hogben. 1957. Absorption of drugs from the stomach. I. The rat. J. Pharmacol. Exptl. Therap. **120:** 528.
21. Schanker, L. S. & C. A. M. Hogben. 1957. Absorption of drugs by the human stomach. Federation Proc. **16:** 334.
22. Schanker, L. S., D. J. Tocco, B. B. Brodie & C. A. M. Hogben. 1958. Absorption of drugs from the rat small intestine. J. Pharmacol. Exptl. Therap. **123:** 81.
23. Hogben, C. A. M., D. J. Tocco, B. B. Brodie & L. S. Schanker. 1959. On the mechanism of intestinal absorption of drugs. J. Pharmacol. Exptl. Therap. **125:** 275.
24. Schanker, L. S. 1959. Absorption of drugs from the rat colon. J. Pharmacol. Exptl. Therap. **126:** 283.
25. Gold, H. & J. Travell. 1934. Ethyl alcohol and strychnine antagonism. J. Pharmacol. Exptl. Therap. **52:** 30.
26. Haag, H. B. 1940. The physiologic activity of cigarette smoke solutions as related to their nicotine content. J. Lab. Clin. Med. **25:** 610.
27. Brawley, R. E. 1935. Studies of the *p*H of normal resting saliva. I. Variations with age and sex. J. Dental Research. **15:** 55.
28. Brawley, R. E. 1935. Studies of the *p*H of normal resting saliva. II. Diurnal variation. J. Dental Research. **15:** 79.
29. Sullivan, H. R., G. Charlton & D. Lennon. 1956. Studies of the hydrogen ion concentration in the mouth. I. Hydrogen ion concentration of normal tooth and tissue surfaces. Australian Dental J. **1:** 273.

ABSORPTION OF NICOTINE UNDER VARIOUS CONDITIONS OF TOBACCO USE

P. S. Larson

Department of Pharmacology, Medical College of Virginia, Richmond, Va.

Cigarette Smoking

Of prime importance in the determination of the degree of absorption of nicotine in smoking is an accurate estimate of the amount of nicotine entering the mouth. An appreciation of this problem may be gained from data reported by Bradford *et al.* (1937). Using a smoking machine set to draw a 35-ml. puff of two seconds' duration once a minute, until two-thirds of a cigarette had been consumed, and collecting both the puffed (mainstream) smoke and the sidestream smoke, these authors found that about 22 per cent of the nicotine in the tobacco burned is transferred to the puffed smoke, about 35 to 38 per cent appears in the sidestream, about three to six per cent condenses in the butt and, by difference, about 37 per cent is pyrolized to other products. Even under constant machine smoking conditions, such factors as moisture content, tightness of pack, and burning quality of the tobacco can influence these distributions. Changing the smoking conditions markedly affects the transfer; thus increasing the puff frequency to one puff per one-half minute increases the mainstream nicotine content by 20 per cent and increasing the puff volume from 25 ml. to 45 ml. increases it by about 27 per cent (Rayburn, 1950).

With the aid of a smoking machine, two approaches can be made toward arriving at an estimate of the amount of nicotine entering the mouth. One is to set the conditions of machine smoking, determine the nicotine transfer into the puffed smoke, and then have the subject duplicate as closely as possible the machine conditions using the same brand of tobacco. The second approach is to determine the individual subject's smoking habits, such as puff volume, puff duration, and negative pressure applied (time between puffs and butt length being fixed) and to duplicate these on a machine. A necessary additional step in both cases is the collection and measurement of all exhaled nicotine, the difference between this and estimated intake representing the amount retained and presumably absorbed.

The following investigators have in general followed the first approach with varying degrees of perfection that are difficult to evaluate: Biederbeck (1908), Lehmann (1908, 1909), Winterstein and Aronson (1928), Wenusch (1927–1928), Pyriki (1932, 1943), Laskowski (1951) and Greenberg *et al.* (1952). Their findings are summarized in TABLE 1. Considering both the procedural and human variables involved, these data are, in general, in quite good agreement.

Hillsman (1950) appears to be the only investigator who used the second approach, his findings having been presented in a paper read at the Fourth Tobacco Chemists Conference held at Pennsylvania State University, University Park, Pa. He has kindly granted permission to reproduce representative data from his report and these are embodied in TABLE 2. The values found are in agreement with much of the data in TABLE 1, and the close correspondence of duplicate determinations in individual subjects attest to the merit of the technique used.

Heinz (1923) and Pierce (1937, 1941) determined the nicotine content of the mainstream smoke by having subjects draw puffs of smoke with nicotine traps interposed between the cigarette and the subject. Using values thus obtained and subtracting from them the amounts exhaled and trapped in ordinary puffing or inhalation of smoke, percentages of absorption were derived. Heinz gave values of 17.5 per cent for puffing and 88 per cent for in-

TABLE 1

ABSORPTION OF NICOTINE IN CIGARETTE SMOKING

Author	Per cent absorption of nicotine from cigarette mainstream smoke on	
	Puffing	Inhaling
Biederbeck, 1908	*ca.* 27	
Lehmann, 1908, 1909	*ca.* 10	*ca.* 80
Winterstein & Aronson, 1928	2.5–11.7	8–32 (with 2 sec. inhalation) 100 (with prolonged inhalation)
Wenusch, 1927–28	*ca.* 8	*ca.* 50 (with normal inhaling) *ca.* 85 (with excessive inhaling)
Pyriki, 1932	4–5	84–95
Pyriki, 1943	*ca.* 5	*ca.* 66 (weak inhaling) 68–76 (moderate inhaling) 84–95 (deep inhaling)
Laskowski, 1951		67
Greenberg, Lester & Haggard, 1952	8–18 (moist cig.) 26–45 (dry cig.)	96–98 (moist cig.) 96–97 (dry cig.)

TABLE 2

ABSORPTION OF NICOTINE IN CIGARETTE SMOKING*

Subject	Total nicotine entering mouth as determined by machine smoking†	Nicotine in smoke expelled by smoker†		Per cent absorbed	
		Inhaled	Uninhaled	Inhaled	Uninhaled
A	2.47 2.40 2.44 av.	0.144 0.254	2.11 2.16	94.1 89.6	13.5 11.5
B	2.28 2.40 2.34 av.	0.040 0.011	1.96 1.99	89.3 99.5	16.2 15.0
C	2.29 2.40 2.35 av.	0.159 0.200	2.10 2.03	93.2 91.5	10.6 13.6
D	2.28 2.40 2.34 av.	0.603 0.860	1.97	74.2 63.2	15.8

* Courtesy of O. L. Hillsman.

† Milligram per cigarette.

haling, both in good agreement with values cited in the tables. Pierce gave values of 78.5 per cent for puffing and 95 per cent for inhaling, of which the puffing value appears unaccountably high.

Bodnar *et al.* (1935) had subjects smoke cigarettes of known nicotine content and collected and analysed their exhaled smoke, as well as the sidestream smoke and material left in the holder, for nicotine content. Assuming that no nicotine was destroyed by combustion, the product of subtraction of these values from the nicotine content of the cigarette was assigned as having been absorbed. On this erroneous basis, 60 per cent absorption was claimed for noninhalers and 93 per cent for inhalers.

Baumberger (1923*a*) found that an average of 66.7 per cent of the particulate matter in cigarette smoke was retained in the mouth on puffing and 88.2 per cent on inhaling. This led him (1923*b*) to assume that equal percentages of nicotine would be retained. Since the nicotine in smoke is transported on or in the smoke particles, this assumption has reasonable merit, leaving the impression that something was abnormally different about the manner in which these subjects puffed.

In addition, it is of interest to cite a value arrived at by an entirely different method by Chalmers and Lewis (1951). Comparison of the antidiuretic effect of smoking with that of nicotine infusions suggested to these authors that the smoker derived 1.0 to 1.5 mg. of nicotine from one cigarette, provided he inhaled vigorously. From the other data presented, this would be of the order expected from a cigarette of moderate nicotine content.

Finally, some experiments by Biederbeck and by Willke (cited by Biederbeck) are of interest. In studies on nicotine vapors drawn into the mouth and held for a few seconds, both investigators found high values for retention, up to 100 per cent. This led Biederbeck as well as Lehmann (1909) to the conclusion that absorption of nicotine from smoke is poorer than from air. A probable explanation is that nicotine in smoke exists as salts rather than as the free base.

Cigar Smoking

According to Winterstein and Aronson, the amount of nicotine absorbed by the smoker from cigars without inhaling was 13 to 20 per cent of the total nicotine in the tobacco smoked. They concluded that more nicotine is absorbed from cigar smoke, even without inhaling, than from cigarette smoke.

Greenberg *et al.* reported that smoking a dry cigar without inhalation resulted in 18 per cent retention of mainstream nicotine by the body, whereas similarly smoking a moist (11 per cent moisture content) cigar resulted in only four per cent retention. When either a dry or a moist cigar was inhaled, the nicotine retention rose to 96 to 97 per cent.

Wenusch (1942) stated that retention from cigar smoke is harder to estimate than that from cigarette smoke, due to varying alkalinity of cigar smoke and the varying degrees of filtration through the unsmoked portion. A cigar with one per cent nicotine and strongly alkaline smoke was said to deposit three times as much nicotine in the mouth as a similar cigar with only a weakly alkaline mainstream smoke; from a medium strength cigar 121 mm. in length there is deposited in the mouth 0.2 mg. nicotine from the first 37 mm., 1 mg. from the second 37 mm., and 3.1 mg. from the third 37 mm. Wenusch concluded that in cigarette smoking, the amount of nicotine absorbed depends on the degree of inhalation, whereas with cigar smoking it depends on the alkalinity of the mainstream smoke and the length of the unsmoked stump.

Pipe Smoking

We have found no quantitative data concerning nicotine absorption in pipe-smoking. One may suppose that a deterent factor to such data collection is the utter lack of a standardized bowl and stem and the wide variability in transfer of nicotine to the mainstream smoke that would result from variations in tightness of packing.

Tobacco Chewing

Gaede (1941) studied nicotine absorption from chewing tobacco employing a chew of 0.6 gm. containing on the average 15 mg. of nicotine. After one hour of chewing, the nicotine content of the residue was reduced by 33 per cent; at two hours, by 50 per cent; at four hours, by 60 per cent; and at eight hours, by 90 per cent. This was said to be a less rapid absorption, even in the early period, than occurs in smoking with inhalation.

In 21 habitual tobacco users who chewed 15.6 to 58.4 gm. of tobacco during 6.5 to 8 hours, Wolff and Giles (1950) found the amount of nicotine absorbed ranged from 8 to 87.7 mg.

Snuff Taking

Gaede (1944) stated that the daily dose of nicotine obtained by the chewer of snuff is from 20 to 60 mg. Presumably, this too would depend upon individual habit.

Summary

Nicotine is absorbed into the body from all forms of tobacco use. In smoking, the efficiency of this absorption varies directly with the extent of the respiratory tract exposed and the duration between inhalation and exhalation. Efficiency approaching 100 per cent appears possible. In tobacco chewing and snuff taking, 100 per cent efficiency in absorption likewise appears possible, subject to such variables as length of retention of the quid, vigor of chewing, and frequency of expectoration.

References

Baumberger, J. P. 1923*a*. The amount of smoke produced from tobacco and its absorption in smoking as determined by electrical precipitation. J. Pharmacol. Exptl. Therap. **21:** 47–57.

Baumberger, J. P. 1923*b*. The nicotine content of tobacco smoke. *Ibid.* **21:** 35–46.

Biederbeck, J. 1908. Neue Untersuchungen über die Absorption von Nikotin und Ammoniak aus Luftmischungen und dem Zigarettenrauch. Inaugural Dissertation. F. Staudenraus. Wurzburg, Germany. : 24.

Bodnar, J., V. L. Nagy & A. Dickmann. 1935. Über die Nicotinaufnahme des Organismus beim Rauchen und das Schicksal des aufgenommenen Nicotins. Biochem. Z. **276:** 317–322.

Bradford, J. A., E. S. Harlow, W. R. Harlan & H. R. Hammer. 1937. Nature of cigaret smoke volatile bases and acids. Ind. Eng. Chem. **29:** 45–50.

Chalmers, T. M. & A. A. G. Lewis. 1951. Stimulation of the supraoptical-hypophyseal system in man. Clin. Sci. **10:** 127–135.

Gaede, D. 1941. Über die Nikotinaufnahme aus Kautabak. Arch. Exptl. Pathol. Pharmakol. Naunyn-Schmiedeberg's. **197:** 72–77.

Gaede, D. 1944. Zur Wirkung des Schnupftabaks. *Ibid.* **203:** 130–145.

Greenberg, L. A., D. Lester & H. W. Haggard. 1952. The absorption of nicotine in tobacco smoking. J. Pharmacol. Exptl. Therap. **104:** 162–167.

Heinz, R. 1923. Ueber die Giftigkeit des Tabakrauches, inbesondere des Zigarettenrauches. Deut. med. Wochschr. **49:** 318–319.

Hillsman, O. L. 1950. The absorption of nicotine by the cigarette smoker. Presented at the 4th Tobacco Chemists Conference, Pennsylvania State University. University Park, Pa. Sept. 12.

Laskowski, K. 1951. Components of tobacco smoke and their absorption in the respiratory system of the smoker. (In Polish). Roczniki Państwowego Zakładu Hig. **2:** 139–160.

Lehmann, K. B. 1908. Untersuchungen über das Tabakrauchen. Münch. med. Wochschr. **55:** 723–725.

Lehmann, K. B. 1909. Chemische und Toxikologische Studien über Tabak, Tabakrauch und das Tabakrauchen. Arch. Hyg. Münch. **68:** 319–420.

Pierce, I. H. 1937, 1941. The absorption of nicotine in cigarette smoking. J. Pharmacol. Exptl. Therap. **60:** 114–115; J. Lab. Clin. Med. **26:** 1322–1325.

Pyriki, C. 1932. Über die Aufnahme des Nicotins beim Zigarettenrauchen. Z. Untersuch. Lebensm. **64:** 163–171.

Pyriki, C. 1943. Über die Nicotinaufnahme des menschlichen Organismus bei Zigarettenrauchen unter besonderer Berucksichtung harzreicher und hartzarmer tabake. Z. physiol. Chem. Hoppe-Seyler's. **277:** 233–247.

Rayburn, C. H. 1950. Influence of certain variables on the composition of cigarette smoke. Presented at the 4th Tobacco Chemists Conference. Sept. 12. Pennsylvania State University. University Park, Pa.

Wenusch, A. 1927–1928. Nikotinmenge im Tabakrauch und Nikotinaufnahme durch den menschlichen Organismus beim normalen (intermittierenden) Rauchen. Fachliche Mitt. Osterr. Tabakregie. **3:** 8–11.

Wenusch, A. 1942. Über die absoluten Mengen des beim Rauchen aufgenommen Nikotins. Chem. Z. **66:** 254–255; Forsch. u. Fortschr. **18:** 77–78.

Winterstein, A. & E. Aronson. 1928. Beiträge zur Kenntnis des Tabakgenusses. II. Mitteilung. Über den Verbleib des Nicotins beim Tabakrauchen. Z. Hyg. **108:** 530–553.

Wolff, W. A. & W. E. Giles. 1950. Studies on tobacco chewing. Federation Proc. **9:** 248.

THE EXCRETION AND METABOLISM OF NICOTINE

Herbert McKennis, Jr.
Department of Pharmacology, Medical College of Virginia, Richmond, Va.

The wide distribution of nicotine in plant material described by Dawson *et al.* elsewhere in these pages, the absorption of nicotine from the smoking of tobacco, as discussed by Larson in another paper in this monograph, the employment of nicotine in diagnostic procedures for diabetes insipidus,[1,2,3] and the use of nicotine as an insecticide, provide ample opportunity for exposure to the alkaloid.

Following the isolation of nicotine from *Nicotiana tabacum* in 1828, the compound has enjoyed many years of use by pharmacologists. Its actions have been studied in a variety of organisms and in a variety of systems. In particular, nicotine has been utilized as a tool in the study of the autonomic nervous system. Despite the number of investigations of the biological action of nicotine little is known concerning the detail of the events responsible for terminating the effects of the compound. Furthermore, there is some possibility that many of the effects that arise following administration of nicotine are in fact effects produced by compounds arising from metabolism of the alkaloid.

Nicotine and related alkaloids have the intriguing combination of a pyridine ring, such as is found in diphosopyridine nucleotide and other important enzymatic constituents, and a pyrrolidine ring that gives it a structural resemblance to the natural amino acids, proline and hydroxyproline. These relationships would in themselves make attractive a study of the mammalian metabolism of nicotine.

The results that I should like to report have been gathered largely with the very valuable collaboration of Lennox B. Turnbull and Edward R. Bowman. Our work has been made possible by the kind financial assistance of the American Tobacco Company, New York, N. Y., the Tobacco Industry Research Committee, also in New York, and the National Institutes of Health, Public Health Service, Bethesda, Md., and finds its roots in the earlier works of many investigators that are briefly mentioned.

It has been observed[4] that as a result of the smoking of tobacco approximately 10 per cent of the absorbed nicotine is excreted unchanged in the urine. Studies in the rat[5] and in the dog[6] have shown that most of the radioactivity of randomly labeled C^{14} nicotine can be recovered in the urine of the animal. In the anesthetized dog receiving nicotine slowly by intravenous infusion, Bennett *et al.*[6] obtained more than 90 per cent of the administered radioactivity in the urine. In similar experiments Owen and Larson[8] subjected concentrated samples of the radioactive urine to paper chromatography. These authors were led to estimate that the metabolism of (−)-nicotine had given rise to 3 major and 4 minor urinary metabolites.

During the past 3½ years studies have been conducted in our laboratory on anesthetized dogs, unanesthetized dogs, rats, and humans. As a result of this work there is emerging a pattern that indicates the involvement of many routes in the metabolism of nicotine. Many of these reactions are unusual and form the foundation for further biochemical and pharmacological studies that may reveal the possible role of the metabolites in inducing or terminating the pharmacological effects arising from administration of the alkaloid.

In a typical experiment on the dog under pentobarbital anesthesia, redistilled (−)-nicotine (total dose of 10 mg./kg.) was infused[7,9] during an 8-hour period into the femoral vein. Bladder urine was collected by an indwelling catheter during administration of the alkaloid and for a subsequent 10-hour period. The acidified urine was then placed upon a column of Dowex 50 (H^+), which served to retain all of the components that give a positive Koenig reaction. These components were then removed from the column by means of aqueous ammonia. A sample of the ammoniacal solution was then subjected to chromatography on paper. The Koenig positive zones were visualized as yellow to red spots by a spray of alcoholic *p*-aminobenzoic acid and subsequent exposure to vapors of cyanogen bromide. In similar paper chromatograms the Koenig positive zones were located as quenching areas in ultraviolet light.

TABLE 1

EXCRETION OF KOENIG POSITIVE COMPOUNDS BY A MALE MONGREL DOG (15.3 KG.) FOLLOWING INTRAVENOUS ADMINISTRATION OF (−)-NICOTINE (153 MG.)

R_f values* of metabolites in Dowex 50 eluate	Calculated mole % of dose†
(1) 0.15	9.21
(2) 0.38	5.41
(3) 0.46	0.88
(4) 0.61	2.79
(5) 0.73	2.84

* Obtained at room temperature (1 vol. *N*/2 ammonia water, 1 vol. 95 per cent ethanol, 4 vol. 1-butanol) on Whatman No. 1 paper by descending method. Control urines were devoid of Koenig positive zones. For spectroscopic determinations zones were localized as quenching area in ultraviolet light. R_f values were generally consistent to ±0.05 units.

† Calculated from optical density of extracts of zones in 0.1 *M* HCl—95 per cent ethanol at 260 mμ—optical density of extract from metabolite-free paper, using ε4820.[22]

The separate zones were then extracted with alcohol. The optical density of the acidified extracts at 260 mμ (TABLE 1) made possible an estimate that approximately 21 per cent of the administered nicotine had been accounted for as metabolites in the Koenig positive areas.

Of immediate interest was the zone at R_f 0.15 that corresponded in R_f value to (+)-γ-(3-pyridyl)-γ-methylaminobutyric acid, which had been prepared[10] by us synthetically from (−)-nicotine and had been previously considered by Larson and his co-workers[11,12] as a possible metabolite of nicotine. Procedures were then devised to deny or confirm this apparent identity of the metabolite and γ-(3-pyridyl)-γ-methylaminobutyric acid that was then indicated only from the R_f values obtained by paper chromatography in a variety of solvent systems.

By extraction with chloroform, unchanged nicotine and other Koenig positive constituents (R_f 0.61 and R_f 0.74, ammonia-ethanol-butanol) were removed from ammoniacal eluate of the Dowex 50 (H^+) column. The ammoniacal solution was then applied to a column of Dowex 1 (OH^-), which retained Koenig positive material with R_f 0.15. The latter material was removed from the column with acetic acid. The residue from removal of the solvent was heated to 155° C. in an atmosphere of nitrogen. A chloroform extract of the thermally treated material contained (−)-cotinine that was formed as a result of lactamization of (+)-γ-(3-pyridyl)-γ-methylaminobutyric acid. The cotinine was identified in the form of its monopicrate, m.p. 104 to 106° C. This identification provided evidence for the conclusion that (+)-γ-(3-pyridyl)-γ-methylaminobutyric acid was in fact present in the urine following administration of (−)-nicotine. It was interesting to note that the cotinine thus obtained has essentially the same optical rotation as cotinine prepared chemically from (−)-nicotine via dibromocotinine. Later experiments in which this (−)-cotinine was reduced[13] with lithium aluminum hydride to (−)-nicotine clearly showed that in the metabolism of (−)-nicotine to (+)-γ-(3-pyridyl)-γ-methylaminobutyric acid the absolute configuration of (−)-nicotine is retained.

TABLE 2

KOENIG POSITIVE METABOLITES EXTRACTED FROM URINE OF SMOKER, NONSMOKER AND DOG FOLLOWING ADMINISTRATION OF NICOTINE

Urine source*	*R_f* values	
	ammonia-ethanol-butanol[10]	*sec*-butanol-formic acid-water[23]
Human smoker	0.61,† 0.74,‡ 0.85,§ 0.90	0.21,† 0.37‡
Human nonsmoker	0.61,† 0.74‡	0.21,† 0.37‡
Dog	0.61,† 0.74,‡ 0.85§	0.21,† 0.37‡

* Adult male human subject received 3 mg./hr. in 10 hourly doses over a 3-day period (total dose 90 mg.). Extract obtained from combined 24-hr. urine samples. Smokers' urine collected as daytime contributions from male laboratory workers. Urine from bladder of mongrel dog collected by indwelling catheter during 8-hr. period of nicotine infusion (10 mg./kg. total dose) and subsequent 10-hr. period. Chloroform extracts of control urines showed no Koenig positive zones.

† Cochromatographed with desmethylcotinine. Acetylation of fraction gave components corresponding in R_f value to acetoxycotinine and desmethylcotinine.

‡ Cochromatographed with cotinine.

§ Cochromatographed with nicotine.

In urine or aqueous solution[8] (+)-γ-(3-pyridyl)-γ-methylaminobutyric acid produces in the presence of cyanogen bromide (without added aromatic amine) a pink color. It is thus responsible, in part at least, for the characteristic color reaction[11] of the urine of dogs after administration of nicotine. The urine of smokers[14] and of nonsmokers[15] after oral administration of (−)-nicotine also gives a characteristic pink color with cyanogen bromide. Chromatograms of the urine of smokers and the urine of one male nonsmoker, who received 90 mg. of (−)-nicotine orally in divided doses over a 3-day period, are quite similar to those of the dog (TABLE 2). The chloroform-soluble nicotine metabolites of the human and, especially, the dog have been subjected to a rather extensive investigation. Progress on the chemical identification of these components was facilitated by the availability of desmethylcotinine and other pyridine compounds, which had been prepared by us[10] prior to the development of procedures for the isolation and identification of metabolites.

Purification of the chloroform-soluble metabolites has been achieved in a variety of ways. In typical experiments the metabolites were dissolved in water after evaporation of the chloroform. The aqueous solution was placed on a column of Dowex 1 (OH^-) that removed an amphoteric component. The effluent from the column was evaporated to dryness. By chromatography on alumina with methanolic ether, Koenig positive fractions (R_f 0.61, 0.74) were obtained. The metabolite in the fraction with R_f 0.74 was obtained as a light-colored oil, representing approximately 10 per cent of the dose of (−)-nicotine administered to the human. The metabolite cochromatographed with cotinine has been identified by preparation of both the dipicrate and monopicrates, which have melting points and infrared spectra in agreement with the authentic material. The optical rotation of this metabolic cotinine is again in agreement with synthetic (−)-cotinine, and indicates that the absolute optical configuration of (−)-nicotine has been retained during the metabolic oxidation of the pyrrolidine ring. Hucker *et al.*[16] have recently obtained cotinine from the metabolism of nicotine *in vitro*, using a fortified preparation from rabbit liver. Experiments in both the dog[13] and the human[17] indicate that cotinine has a key role in the metabolism of nicotine. In both species administration of (−)-cotinine or (−)-nicotine leads to the excretion of chloroform-soluble material with R_f 0.61. This component has the properties of a mixture which, in the case of the dog, has been separated into two crystalline components.

Following treatment with acetic anhydride in the presence of pyridine, the component with R_f 0.61 was rechromatographed on alumina.[13] The eluates from the alumina column yielded crystalline (−)-desmethylcotinine, m.p. 135 to 136° C., identified by comparison with an authentic sample,[13] R_f 0.61, and an acetoxy compound, R_f 0.75, which was converted to a crystalline picrate, m.p. 166.5 to 170° C. The elementary analysis, corresponding to $C_{18}H_{17}N_5O_{10}$, served to indicate that (−)-nicotine had been metabolized via (−)-cotinine to hydroxycotinine, $C_{10}H_{12}N_2O_2$. The hydroxyl group was provisionally assigned to the pyridine ring on the basis of the strong positive Koenig reaction of the compound.

Studies on the chloroform-soluble amphoteric metabolite that can be eluted from Dowex 1 (OH^-) by acid or large quantities of water indicate its structure to be γ-(3-pyridyl)-β-oxo-N-methylbutyramide. The metabolite has been obtained in crystalline form, m.p. 114 to 116° C. Upon hydrolysis in aqueous acid or base it yields methylamine, identified in the form of the picric acid salt, and a keto acid with an empirical formula of $C_9H_9NO_3$ corresponding to a γ-(3-pyridyl)oxobutyric acid. The melting point data indicated that this compound was different from γ-(3-pyridyl)-γ-oxobutyric acid.[10,18] The position of the side chain in the indicated 3-position was established by a Wolf-Kishner reduction of the metabolite to γ-(3-pyridyl)butyric acid that was identified by comparison with authentic γ-(3-pyridyl)butyric acid, prepared by reduction of γ-(3-pyridyl)-γ-oxobutyric acid. Both the metabolite and the keto acid obtained by hydrolysis of the metabolite yielded oximes. The oximino acid was subjected to a Beckmann rearrangement that afforded 3-pyridylacetic acid and indicated that the oxo group of the metabolite was in the β-position.

The isolation and characterization of γ-(3-pyridyl)-γ-oxo-*N*-methylbutyramide as a result of the metabolism of (−)-nicotine suggest immediately that this metabolite could serve as a precursor of methylamine in the body. Werle and Meyer[19] reported that the incubation of liver slices with nicotine led to the production of methylamine. Hydrolysis of the oxo amide *in vivo* would result in the formation of methylamine and γ-(3-pyridyl)-β-oxobutyric acid. Although a derivative of acetoacetic acid, this latter compound shows a marked stability to both acid and base. These facts suggest that if hydrolysis of the oxo acid occurs to a great extent *in vivo* the reaction would be primarily dependent upon the presence of enzyme systems. A ketonic hydrolysis would lead to 3-pyridylacetone, whereas an acid hydrolysis would yield 3-pyridylacetic acid. Chromatograms of dog urine following administration of either (−)-nicotine or (−)-cotinine gave evidence that strongly suggested the presence of 3-pyridylacetic acid, which has recently been isolated from the urine in crystalline form. Experiments to determine whether or not γ-(3-pyridyl)-β-oxobutyric acid is the immediate precursor of 3-pyridylacetic acid are in progress.

Nicotine, in addition to undergoing oxidative changes in the pyrrolidine ring leading to the production of the foregoing, can serve in the body, in common with many other pyridine compounds, as a methyl acceptor. Following administration of (−)-nicotine to the dog, evidence has been obtained[20] that suggests the presence in the urine of several quaternary ammonium compounds, in addition to the anticipated isomethylnicotinium compound, which arise as a result of metabolism of the alkaloid. Following removal of chloroform-soluble metabolites by extraction, and acidic components by Dowex 1 (OH^-), the processed urine of the dog was treated with ammonium Reineckate. The insoluble Reineckate was then converted to the soluble iodides from which nicotine isomethoiodide was obtained by fractional crystallization. The identity of the compound was confirmed by comparison with an authentic sample obtained by treating nicotine with methyliodide. The total yield of the methoiodide from one dog accounted for 13 per cent of the administered dose of (−)-nicotine. The possibility remains that in addition to nicotine many of its metabolites can undergo a similar methylation in the body. Following administration of (−)-cotinine alone to a dog, 40 per cent of the administered dose was isolated from the urine as the methonium derivative.

Studies such as the foregoing, particularly where synthetic methods are available for the production of adequate quantities of metabolites, enable the investigator to proceed with the important task of finding the role that these metabolites may play in the total physiological response of the organism to nicotine, and the functions that the metabolites may have in terminating and controlling the response to nicotine.

Both (−)-cotinine and (−)-desmethylcotinine have been found[21] devoid of the pressor activity associated with the precursor nicotine. In large doses (approximately 100 mg./kg.) these metabolites were found to exert a depressor effect that was not abolished by atropine (unpublished observations by J. F. Borzelleca, E. R. Bowman, and myself). It is apparent that in undergoing metabolic oxidation to cotinine, nicotine has lost much of its pharmacological activity. For comparative purposes it is interesting to note that we found the LD_{50} of (−)-nicotine to be 10.3 mg./kg., intraperitoneally in mice, whereas

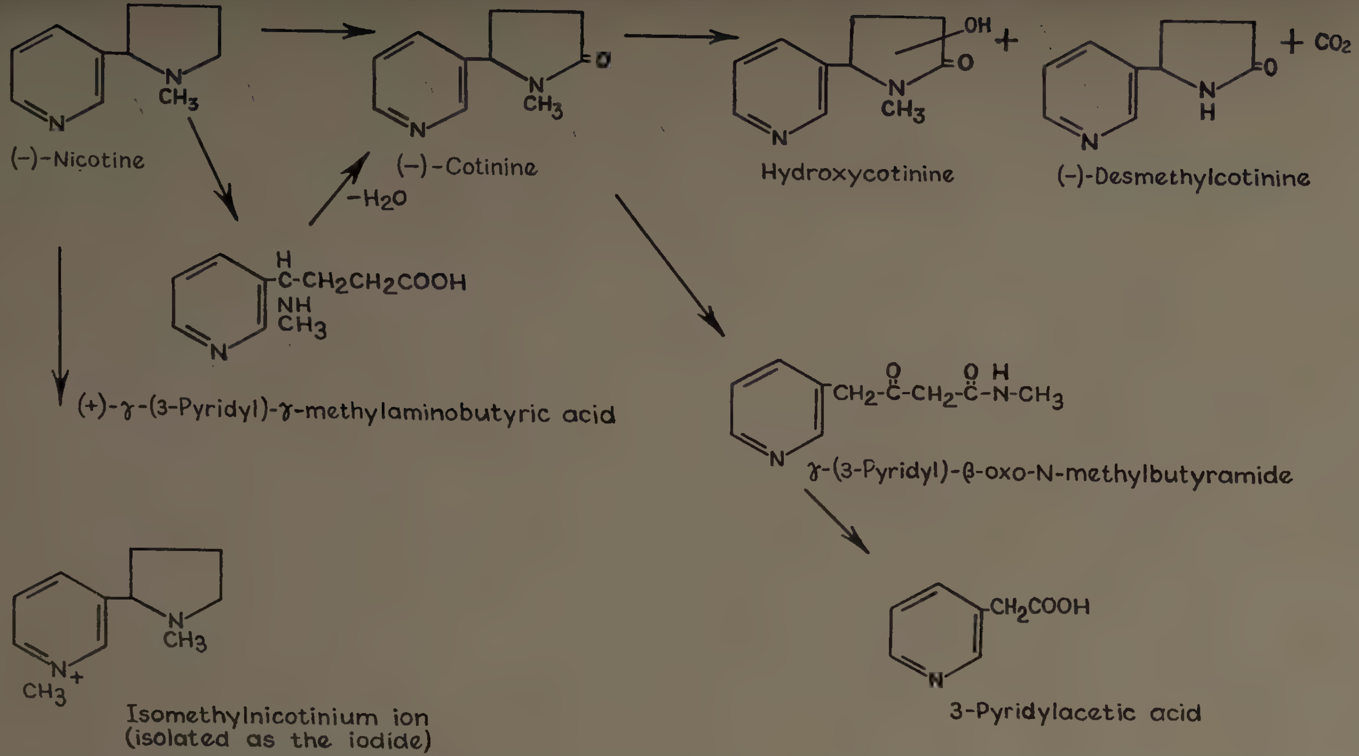

FIGURE 1. Schematic presentation of compounds that have been isolated as a result of mammalian metabolism of (−)-nicotine. Although γ-(3-pyridyl)-γ-methylaminobutyraldehyde has been considered as a possible precursor of this acid,[24] the aldehyde has not been isolated and chemically identified. The possibility that a cyclized form of the aldehyde, 5-(3′-pyridyl)-2-hydroxy-1-methylpyrrolidine, participates as an intermediate in nicotine metabolism has been considered in early[25] and more recent work.[16]

that of (−)-cotinine is in excess of 930 mg./kg. The human subject has tolerated 300 mg. of cotinine in a single dose without evidence of discomfort (unpublished observations by myself and E. R. Bowman).

Pharmacological experiments have indicated that the metabolic oxidation of (−)-nicotine to (−)-cotinine leads to a dramatic loss of activity. A full exploration of the pharmacological effects of (−)-cotinine and other metabolites is required to determine the composite role of these substances in the physiological response of the organism to nicotine. Similarly, much investigation is required to complete and extend our understanding of the metabolism of the parent alkaloid. The known relationships of the mammalian metabolites are schematically summarized in FIGURE 1.

References

1. Chalmers, T. M. & A. A. Lewis. 1951. Clin. Sci. **10:** 137.
2. Cates, J. E. & O. Garrod. 1951. Clin. Sci. **10:** 145.
3. Dingman, J. F., K. Benirschke & G. Thorn. 1957. Am. J. Med. **23:** 226.
4. Corcoran, A. C., O. M. Helmer & I. Page. 1939. J. Biol. Chem. **128:** 89.
5. Ganz, A., F. E. Kensey & E. M. K. Geiling. 1951. J. Pharmacol. Exptl. Therap. **103:** 209.
6. Bennett, D. R., R. E. Tedeschi & P. S. Larson. 1954. Arch. intern. Pharmacodynamie. **98:** 221.
7. McKennis, H., Jr., L. B. Turnbull & E. R. Bowman. 1957. J. Am. Chem. Soc. **79:** 6342.
8. Owen, F. B., Jr. & P. S. Larson. 1958. Arch. intern. Pharmacodynamie. **115:** 402.
9. McKennis, H., Jr., L. B. Turnbull & E. R. Bowman. 1958. J. Am. Chem. Soc. **80:** 6597.
10. McKennis, H., Jr., L. B. Turnbull, H. N. Wingfield, Jr. & L. J. Dewey. 1958. J. Am. Chem. Soc. **80:** 1634.
11. Larson, P. S. & H. B. Haag. 1942. J. Pharmacol. Exptl. Therap. **76:** 240.
12. Larson, P. S., H. B. Haag & J. K. Finnegan. 1946. J. Pharmacol. Exptl. Therap. **86:** 239.
13. McKennis, H., Jr., L. B. Turnbull, E. R. Bowman & E. Wada. 1959. J. Am. Chem. Soc. **81:** 3951.
14. Werle, E., H. Schievelbein & D. Spieth. 1956. Arzneimittel-Forsch. **6:** 322.
15. Bowman, E. R., L. B. Turnbull & H. McKennis, Jr. 1959. J. Pharmacol. Exptl. Therap. **127:** 92.
16. Hucker, H. B., J. R. Gillette & B. B. Brodie. 1959. Nature. **183:** 47.
17. McKennis, H., Jr. & E. R. Bowman. 1959. Abstracts of Papers, 13th Tobacco Chemists' Research Conference. : 14. Lexington, Ky. October 29–30.
18. Castle, R. N. & A. Burger. 1954. J. Am. Pharmacol. Assoc. Sci. Ed. **43:** 163.
19. Werle, E. & A. Meyer. 1950. Biochem. Z. **321:** 221.
20. Turnbull, L. B., E. R. Bowman & H. McKennis, Jr. 1960. Federation Proc. **19:** 268.
21. Bowman, E. R., H. G. Kennedy, Jr., E. Wada & H. McKennis, Jr. 1959. Abstracts of Communications. 21st Intern. Congr. Physiol. Sci. : 41. Buenos Aires, Argentina. August 9–15.
22 Swain, M. L., A. Eisner, C. F. Woodward & B. A. Brice. 1949. J. Am. Chem. Soc. **71:** 1341.
23. Hausmann, W. 1952. J. Am. Chem. Soc. **74:** 3181.
24. Frankenburg, W. G., A. M. Gottscho & A. A. Vaitekunas. 1955. Proc. 1st Intern. Sci. Congr. on Tobacco. **2:** 419.
25. Tedeschi, R. E. 1953. Thesis. Medical College of Virginia. Richmond, Va.

Part II. Pharmacological Actions of Nicotine and Tobacco Smoke

COMPONENTS OF PHARMACOLOGIC INTEREST IN TOBACCO SMOKE

C. J. Kensler
Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, Mass.

Before attempting to indicate briefly the types of components of potential pharmacological interest in tobacco smoke, I should like to discuss pharmacological activity. This term applies to the activity of materials or compounds that produce a detectable and, preferably, measurable effect on man or some other biological system. In the case of man or other animals, this effect may be beneficial, of no consequence, or harmful. These effects may be on the tissue at the site of application or exposure, or on a tissue or organ system remote from this site. The effects may be immediate or delayed in onset and may be of short or long duration. The effects, however, will be dependent on the effective dose and on the dose schedule. By effective dose is meant the amount of agent not only delivered but retained, which may represent a small or large percentage of the material present in the smoke.

All compounds, if administered to man or other animals in a sufficiently large amount, would produce a pharmacologic effect, specific or nonspecific, and the converse, in so far as this has been established, is also true: namely, that all compounds can be administered in low doses that do not produce any observable effect. This emphasizes the need for quantitative data as to the amounts of various compounds in smoke. Also, it is important when considering the actions of components of smoke to remember that smoke is a complex mixture and that the effects of a single component by itself may be masked or altered by the actions of other components of the mixture. The pharmacological actions of tobacco smoke and specific components of interest have been reviewed previously (Haag and Larson, 1944; Kensler, 1954).

Tobacco smoke as a physical entity consists of two phases, the gas phase and the particulate phase. As a physical entity, cigarette smoke displaces an equal volume of air and introduces a particulate phase consisting of droplets, many of one micron or smaller size. Droplets or particles of this size can produce effects such as decreasing airway conductance (that is, increasing airway resistance) when inhaled. Nadel *et al.* (1959) have reported that smoking a cigarette produces a 50 per cent decrease in airway conductance or, conversely, the airway resistance is doubled. This, however, is a small change, as it takes four- to fivefold increases in resistance to produce dyspnea, and many asthmatics have a 10- to 20-fold increase. This effect of tobacco smoke when inhaled would appear to be the result of the physical nature of the material (Dautrebande, 1952) and to be independent of the pharmacologically active components therein.

There are two recent compilations of the available information and references on the chemical components of tobacco smoke, Bentley and Berry's 1959 annotated bibliography of the constituents of tobacco smoke and the review of Johnstone and Plimmer, 1959. From the Johnstone and Plimmer compilation it may be seen that many of the compounds present in tobacco are transferred into the smoke during the combustion process. A summary of these authors' tabular data indicates that there are 202 compounds of various types that have been identified in the tobacco and a larger number, 261, in the smoke. However, roughly two thirds of the compounds present in the smoke have not been found in the plant and, hence, are presumably formed during or subsequent to the combustion process. This information is summarized in TABLE 1. The number of compounds of each class thus far identified in the plant or smoke are listed, and the number of compounds present in both plant and smoke are listed in the last column. For detailed information on the specific compounds identified, the reader is referred to the Johnstone and Plimmer review, as only a few examples can be included in this presentation. The absolute and relative concentrations of several compounds in the smoke have also been found to vary with differences in conditioning of the tobacco and smoking conditions.

TABLE 1

IDENTIFIED COMPONENTS OF TOBACCO AND TOBACCO SMOKE*

	Plant	Smoke	Plant and smoke
Aliphatics	5	18	2
Paraffins (C_{25}–C_{33})	15	14	14
Aromatics	?	60	?
Alcohols and esters	18	19	7
Sterols	5	3	3
Aldehydes and ketones	18	18	7
Acids	38	44	29
Phenols and polyphenols	22	16	4
Alkaloids and other bases	27	38	14
Amino acids and related compounds	26	4	4
Inorganic elements	20	15	14
Misc. (gas, etc.)	8	12	0
Totals	202	261	98

* Compiled from a review by Johnstone and Plimmer, 1959.

Some of the components of the gas phase of tobacco smoke are components of potential interest, and I shall select CO, HCN, and the nitrogen oxides NO–NO_2 for discussion.

Carbon monoxide has been reported to represent approximately 4 per cent of the gas phase of tobacco smoke (Osborne *et al.*, 1956), whereas illuminating gas contains 6 to 30 per cent, and engine exhaust from 1 to 7 per cent. Exposure to 1 per cent concentration of CO is lethal for resting man in 20 to 30 min.—an exercising man succumbs sooner.

We all know that tobacco smokers do not succumb to CO poisoning, despite the lethal concentration of CO in tobacco smoke as measured in a standard puff. CO acts by combining with hemoglobin to form carboxyhemoglobin and interfering with oxygen transport in the blood. Measurements (Hanson and Hastings, 1933) of carboxyhemoglobin in the blood of smokers and nonsmokers have shown that nonsmokers have blood levels of 1 to 2 per cent, while smokers of 10 to 15 cigarettes/day have carboxyhemoglobin levels of 3 to 4 per cent and smokers of 10 pipesful/day show blood levels of 4 per cent. In smokers, measurements made 12 to 18 hours after smoking show carboxyhemoglobin levels of 2 per cent.

It is known that persons with CO blood saturation of up to 30 per cent are usually asymptomatic, while saturations of 30 to 50 per cent lead to headache, confusion and fainting, 50 to 70 per cent causes coma, and 70 per cent is fatal. With this knowledge and the information cited above on carboxyhemoglobin levels, it is quite clear that, despite the high concentration of CO in tobacco smoke, this represents little if any hazard to normal smokers. The reasons for the low carboxyhemoglobin levels in smokers appear to be the dilution of the smoke in lungs and the intermittent nature of the exposure. For example, the volume of the puff is approximately 35 cc., and the volume of the lungs and airways is about 3.5 l. This represents a 100-fold dilution of puff concentration, so that the exposure is to 0.04 per cent CO rather than the 4 per cent in the 35 ml. puff. In addition, the intermittent exposure (2 sec. puff) for the average cigarette smoker with 1 puff/min. means that the exposure time while smoking represents only 3 to 4 per cent of the total time involved.

With respect to HCN, which is a systemic poison that interferes with cellular oxidation, acute fatal doses may be as low as 200 mg. Cigarette smoke has been reported to contain about 50 to 75 γ/30 ml. puff. This would indicate that smokers (2 packs/day) who inhale would inhale a possibly lethal dose of HCN every 10 days.

The major pathway in the metabolism of CN is its conversion to thiocyanate (CNS) by the enzyme rhodanese, which is present in many tissues, particularly of the liver. Therefore CNS blood levels might be expected to give some indication of the CN absorbed. Trasoff and Schneeberg (1944) have reported that habitual smokers do have a slightly elevated blood CNS level of 1.91 mg./100 ml., as opposed to a control nonsmoker level around 1.05 mg./100 ml. In a study of 3 individuals, Trasoff and Schneeberg measured blood CNS levels after 24 hours of abstention from smoking and again after each subject had smoked 30 to 40 cigarettes. No increase in blood CNS was detected. It would therefore appear that the HCN content of tobacco smoke represents little if any hazard to the normal smoker.

With respect to the oxides of nitrogen, the maximal acceptable concentration for long-term exposure has been generally set at 25 ppm. Recently the American Standards Association (Gray, 1959) adopted a figure of 5 ppm of NO_2. Determination of NO and NO_2 in cigarette smoke (Hagen Smit *et al.*, 1959) has indicated concentrations of 150 to 650 ppm, in cigar smoke of 400 to 950 ppm, and in pipe smoke of 125 to 1150 ppm.

As in the case of CO, these concentrations are high with respect to their dangerous concentrations in continuous exposure (Gray, 1959). In rats, for example, pulmonary hemorrhage but no edema resulted from exposure to 43 ppm of NO_2 for 4 hours. In cats no effects were reported following 6 hours exposure to 110 to 125 ppm, but death occurred in one hour at from 340 to 410 ppm. In man it has been reported that at 64 ppm there was moderate irritation of the larynx, at 100 ppm marked irritation and cough and, at 207 ppm, severe irritation of both nose and larynx. NO_2 has also been reported to inhibit ciliary movement in tracheal preparations (Tremer *et al.*, 1959). As no dose response data were reported, it is difficult to evaluate this finding. In my laboratory almost everything applied to rabbit trachea produced stimulation at low doses and inhibition at higher doses, as in the case of nicotine. It is clear, however, that the possible role played by oxides of nitrogen in the effects of tobacco smoke is one that needs further study. The information available at present indicates, however, that NO_2 in tobacco smoke probably does not represent a serious hazard because (1) the reported concentration includes both NO and NO_2, and NO is much less active, (2) the puff concentration would be diluted approximately 100-fold in the lungs, and (3) the exposure is intermittent and of short duration.

Data indicating the chemical complexity of the particulate phase of tobacco smoke have already been presented. Probably more than 80 per cent of the particulate is retained by smokers who inhale deeply and smaller amounts by noninhalers, as indicated by nicotine determinations (according to studies by Larson reported elsewhere in this monograph). In view of the lack of time and, in most cases a lack of definitive pharmacological information, my discussion of particulate components will be limited. With respect to the 38 alkaloids and other bases mentioned earlier, nicotine would appear to be the important pharmacological component. The pharmacology of nicotine in health and disease is discussed in many of the other papers in this symposium.

With respect to the other components, they may have effects at the sites of exposure or, when absorbed, act systemically. Many, if not most, of these compounds are present in amounts that by themselves would be without significant effect. The aliphatics, paraffins, alcohols and esters, aldehydes and ketones, acids, phenols and polyphenols, and sterols fall in this category, although detailed information is lacking. One point that should be emphasized is the report of Larson (1952) that the relative irritant versus edema-producing activity of smoke varies as a function of time from smoke formation to assay and, hence, to be strictly applicable, studies should be conducted on fresh smoke. Free radicals are present, but their importance is unknown.

The highly complex class of aromatic compounds has been shown to contain trace quantities of compounds such as 3,4-benzpyrene, which are carcinogenic for some species of animals by some routes of administration (Cooper *et al.*, 1954; Van Duuren, 1958; Wynder, 1959). The carcinogenic activity of large amounts of condensates of cigarette and cigar smoke has been detected using mouse skin as the test object, but the amounts of identified carcinogenic aromatic hydrocarbons appear to be much too small to explain the mouse skin results. Inhalation experiments to produce cancer by tobacco smoke have been essentially negative in all species so far tested, although changes apparently similar to "carcinoma *in situ*" lesions have been observed to appear in mice after a few months exposure (Leuchtenberger *et al.*, 1958). Even with lifetime exposure these lesions did not develop into cancer. The aromatic hydrocarbons have no known effects on the cardiovascular system.

In conclusion, it should be pointed out that estimation of pharmacological importance of the amounts of the compounds present in tobacco smoke is best related to the response of the average individual, and should not be generalized to include individuals who are extremely sensitive because of genetic or other reasons, or to those individuals who, as a result of differential aging or the presence or history of disease, may differ markedly from the average or normal in their response. Even the estimation of the factors of importance in the average response to tobacco smoke are extremely complex and will require years of careful experimentation before definitive quantitative information is available.

References

Bentley, H. R. & E. G. N. Berry. 1959. The constituents of tobacco smoke. Tobacco Manufacturers' Standing Committee. London, England.

Cooper, R. L., A. J. Lindsey & R. E. Waller. 1954. The presence of 3,4-benzpyrene in cigarette smoke. Chem. & Ind. (London). **46:** 1418.

Dautreband, L. 1952. Physiological and pharmacological characteristics of liquid aerosols. Physiol. Revs. **32:** 214.

Gray, E. L. 1959. Oxides of nitrogen: their occurrence, toxicity and hazard. A.M.A. Arch. Ind. Health. **19:** 479.

Haag, H. & P. S. Larson. 1944. Recent advances in the pharmacology of nicotine. Virginia Med. Monthly. **71:** 235.

Hagen Smit, A. J., M. F. Brunelle & J. Hara. 1959. Nitrogen oxide content of smoke from different types of tobacco. A.M.A. Arch. Ind. Health. **20:** 399.

Hanson, H. B. & A. B. Hastings. 1933. The effect of smoking on the carbon monoxide content of blood. J. Am. Med. Assoc. **100:** 1481.

Johnstone, R. A. W. & J. R. Plimmer. 1959. The chemical constituents of tobacco and tobacco smoke. Chem. Revs. **59:** 885.

Kensler, C. J. 1954. Biologic Effects of Tobacco–Pharmacology. Chapt. 2.: 35–62. Little, Brown. Boston, Mass.

Larson, P. S. 1952. Metabolism of nicotine and the nature of tobacco smoke irri ants. Ind. Eng. Chem. **44:** 279.

Leuchtenberger, C., R. Leuchtenberger & P. Doolin. 1958. A correlated histological, cytological and cytochemical study of the tracheobronchial tree and lungs of mice exposed to cigarette smoke. Cancer. **11:** 490.

Nadel, J. A., D. F. Tierney & J. H. Comroe. 1959. Pulmonary responses to aerosols. Presented to the Third Air Pollution Medical Research Conference. Los Angeles, Calif.

Osborne, J. S., S. Adamek & M. E. Hobbs. 1956. Some components of the gas phase of cigarette smoke. I. Anal. Chem. **28:** 211.

Trasoff, A. & N. Schneeberg. 1944. Naturally occurring blood sulfocyanates and their relation to blood pressure. Am. J. Med. Sci. **207:** 63.

Tremer, H. M., H. L. Falk & P. Kotin. 1959. Effect of air pollutants on ciliated mucous secretory epithelium. J. Natl. Cancer Inst. **23:** 979.

Van Duuren, B. L. 1958. Identification of some polynuclear aromatic hydrocarbons in cigarette smoke condensate. J. Natl. Cancer Inst. **21:** 1.

Wynder, E. L. 1959. Laboratory contributions to the tobacco-cancer problem. Brit. Med. J. **i:** 317.

THE PHARMACOLOGICAL ACTIONS OF NICOTINE*

Julius H. Comroe, Jr.
Cardiovascular Research Institute, University of California Medical Center, San Francisco, Calif.

Nicotine is one of the most widely used drugs in the United States and one of the most fascinating from the physiological point of view. Nevertheless many physicians know little about the specific effects of nicotine in the body and the mechanisms by which it acts; furthermore, many pharmacologists are familiar only with the effects of relatively huge doses applied locally or given intravenously. We are presented, then, with two questions: (1) What *can* nicotine do to *any* system in *any* dosage in *any* species? (2) What *does* nicotine do in man in the doses inhaled in tobacco smoke, in the usual time interval, in an individual accustomed to smoking?

What Can Nicotine Do?

Effects on motor systems. These are by far the best known effects of nicotine. Every physician knows that nicotine, in low concentrations, can stimulate sympathetic ganglion cells and, in high concentration, can paralyze them. The stimulant effect applies to all sympathetic ganglion cells whether they are in the paravertebral ganglionated chain or not. Thus nicotine can cause the adrenal medulla (which in some respects acts as a group of sympathetic ganglion cells) to liberate catecholamines; it can also act on sympathetic ganglion cells (or something resembling them) in the isolated heart and evoke all the signs of marked sympathetic stimulation of the ventricles, including liberation of norepinephrine. By its direct stimulant action on the paravertebral sympathetic ganglia, it can produce all the known effects of sympathetic stimulation on the heart, blood vessels, eye, gastrointestinal tract, bronchioles, and bladder, as well as an increase in metabolic rate, blood sugar, and blood unesterified fatty acids.[1]

These effects are all absent after administration of large doses of tetraethyl ammonium, hexamethonium, and nicotine itself, all of which block the stimulant action of nicotine, of acetyl choline, and of preganglionic nerve impulses on sympathetic ganglion cells.

Less well-known is the fact that nicotine, in low concentration, can also stimulate parasympathetic ganglion cells and, in high concentration, block them. Since in many organs sympathetic and parasympathetic nerve stimulation produce opposite effects, it becomes important to know which system, if any, is stimulated by ordinary doses.

Nicotine can also cause skeletal muscle to twitch (fasciculation) by a stimulant action on the motor end plate, a structure that pharmacologically often behaves similarly to autonomic ganglia. The *stimulant* effect of nicotine on this motor system is neither powerful nor well defined. However, the *depressant* action of high concentrations on neuromuscular transmission is well authenticated; toxic doses of nicotine presumably cause death by peripheral

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neurorespiratory-muscular block, which occurs at a time when the respiratory center is still capable of sending impulses over efferent nerves.[2]

Nicotine can also cause increased beating of cilia in low concentration and paralysis in high concentration (the action of tobacco smoke on bronchial and esophageal cilia should not be confused with the actions of nicotine alone).

Effects on sensory systems. Some physicians realize vaguely that nicotine can stimulate certain sensory receptors but consider this action a pharmacological curiosity. Even eminent pharmacologists have stated: "The rise of blood pressure, of pulse rate and of vasoconstriction in the skin are all the result of the stimulation by nicotine of sympathetic ganglion cells"[3] and "The major action (of nicotine) consists of a primary transient stimulation . . . of sympathetic and parasympathetic ganglion cells."[4] Actually, if evidence obtained in cats and dogs can be applied to man, almost all the actions of nicotine inhaled in tobacco smoke are brought about by the stimulant effect of nicotine on certain chemoreceptors that initiate reflexes affecting many parts of the body.

The most important of these are the chemoreceptors of the carotid and aortic bodies. Although the physiological role of these receptors is to detect changes in the P_{O_2}, P_{CO_2} and *p*H of the arterial blood and to signal the respiratory and vasomotor centers to take appropriate action,[5] they are also stimulated powerfully by minute doses of nicotine and its congeners (lobeline has been used in most physiological investigations). The typical effects are an increase in both the rate and depth of breathing, and circulatory actions typical of sympathetic stimulation, such as vasoconstriction mingled with vasodilatation, cardiac acceleration, and increase in blood pressure.* The effects on the circulation caused by chemoreceptor reflexes (acting through the efferent sympathetic nervous system) and those produced by the direct action of nicotine on sympathetic ganglia are so similar that for decades pharmacologists have attributed all of the circulatory effects of nicotine to direct ganglionic stimulation. However, if one begins with intravenous doses of nicotine (in either cats or dogs) so small that *no* respiratory or circulatory action is produced and then increases the amount to about one μg./kg., one notes small but definite increases in respiration and blood pressure that can no longer be obtained after denervation of the carotid and aortic bodies. In other words, the smallest effective doses do not stimulate sympathetic ganglia directly.[15]

Another group of sensory receptors, in the pulmonary and coronary circulation, is activated in cats and dogs by very low concentrations of nicotine.[7] Activation of these pulmonary and coronary chemoreflexes produces dramatic changes that are exactly the opposite of carotid and aortic body excitation, that is, it produces reflex apnea, bradycardia, vasodilation, and hypotension.[7,8] These effects are produced in cats and dogs by doses in the range of four to eight μg./kg. intravenously. To obtain some concept of the concentration of nicotine required to stimulate sympathetic ganglia directly, one finds that if the carotid bodies, aortic bodies, and thoracic chemoreflexes are made inoperative, approximately 32 μg./kg. of nicotine I.V. are required to cause hypertension, tachycardia and peripheral vasoconstriction. (Incidentally, evidence available at present indicates that parasympathetic ganglia require still higher concentrations for direct stimulation.)

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* M. De Burgh Daly and M. J. Scott[16] (**145**: 440–446, 1959) have emphasized that in the dog, carotid body stimulation leads to hyperpnea but not to tachycardia, and therefore they believe that the chemoreceptors are in no way responsible for reflex stimulation of the sympathetic cardio-accelerator system. However, Comroe[6] showed 20 years previously that while the carotid body of the dog was responsible for most of the reflex hyperpnea of anoxia and nicotine, the aortic body was responsible for most of the reflex hypertension and cardiac acceleration. Together, the carotid and aortic bodies produce hyperpnea tachycardia, and hypertension.

Still other sensory receptors are activated by nicotine. Nicotine causes pacinian corpuscles to discharge;[9] Sarnoff[10] has hypothesized that pacinian corpuscles in the pancreas (and possibly elsewhere) may regulate blood pressure and/or flow in some species. Lobeline (a congener of nicotine) is known to stimulate pain receptors somewhere in the distribution of the pulmonary circulation in man,[11] and there is some evidence of an effect of nicotine on the taste buds of the tongue. Rothman and Coon[12] have demonstrated that very low concentrations of nicotine excite cutaneous receptors and, by an axone reflex, cause local sweating and pilomotor erection. Nicotine may also cause either peripheral vasoconstriction (skin) or vasodilatation (muscle) by an axone reflex.[13] All of the effects mentioned in this paragraph are abolished by prior administration of ganglionic blocking agents or by large doses of nicotine itself.

Effects on the central nervous system. Nicotine is not an analeptic (like metrazol), nor does it produce wakefulness and insomnia (like amphetamine). Nevertheless, it does have subtle effects on man's psyche. Although it is hard to identify the effect, it appears that smoking produces pleasurable reactions or tranquility and that this is due, at least in part, to nicotine, and not entirely to the physical manipulations involved in smoking.[14]

No one has identified all of the various cells in the central nervous system affected by nicotine. It is known that dilute nicotine applied directly to the motor cortex produces convulsions and that concentrated solutions depress these cells. (Incidentally, large doses localized to the carotid body receptors can cause convulsions by reflex action; little is known of the effects of minimal chemoreceptor stimulation on cortical activity.)

It has been shown beyond question that ordinary doses of nicotine can liberate, in some sensitive individuals, antidiuretic hormone from the posterior pituitary,[3] presumably by activation of the supraopticohypothalamic system. In a sense, then, this represents activation by nicotine of another type of chemoreceptor reflex within the hypothalamus-pituitary region; it is not known whether activation of carotid and aortic bodies can activate this system reflexly or whether local application is required.

It is important to remember that liberation of antidiuretic hormone from the posterior pituitary does not mean that pitressin or some substance capable of constricting fine blood vessels is also circulating in effective concentrations; Walker demonstrated many years ago that, although very low concentrations of posterior pituitary hormone cause antidiuresis, approximately 200 times this concentration is needed to constrict blood vessels.

This long and probably incomplete recital of what nicotine *can* do in various concentrations illustrates many possible conflicts in action. Thus its stimulant effects on sympathetic ganglia and the adrenal medulla lead to increased rate and force of cardiac contraction, predominant vasoconstriction, and hypertension, but its stimulant action on parasympathetic ganglia leads to opposite effects. Its actions on the carotid and aortic bodies produce hyperpnea and hypertension; its actions on the thoracic chemoreflexes cause apnea and hypotension. In addition, each important change in respiration or blood pressure initiates secondary, compensatory changes.

The problem of stating with certainty the actions of nicotine in man can be realized when one adds to the above list the known facts of (1) depression by high concentrations of the functions stimulated by low concentration, (2) the tolerance developed by man to long-continued usage of nicotine, (3) the great variability in response shown by normal individuals, and (4) the qualitative change in response following surgical procedures, such as a variety of operations on the sympathetic nervous system.

Other papers in this monograph discuss many measurements following administration in man. As we read them, let us think in terms of dosage or concentration used and dose per unit time, the type of subject (tolerant or novice), route of administration (whether by inhalation or intravenously), and anatomic and pharmacological integrity of sensory and motor autonomic pathway in the individual, for all of these can influence to a marked degree the final picture. Let us also remember that nicotine, in any reasonable dose, acts only through existing physiological pathways, and never acts as a nonspecific tissue poison.

References

1. Havel, R. J. & A. Goldfien. 1959. J. Lipid Research. **1:** 102.
2. Gold, H. & W. Modell. 1936. J. Pharmacol. Exptl. Therap. **57:** 310.
3. Burn, J. H., L. H. Truelove & I. Burn. 1945. Brit. Med. J. **1:** 403.
4. Goodman, L. & A. Gilman. The Pharmacological Basis for Therapeutics. 2nd ed. MacMillan. New York, N. Y.
5. Schmidt, C. F. & J. H. Comroe, Jr. 1940. Physiol. Rev. **20:** 115.
6. Comroe, J. H., Jr. 1939. Am. J. Physiol. **127:** 176–191.
7. Dawes, G. S. & J. H. Comroe, Jr. 1954. Physiol. Rev. **34:** 167.
8. West, J. W., S. V. Guzman & S. Bellet. 1958. Arc. Res. **6:** 389.
9. Brown, G. L. & J. A. B. Gray. 1948. J. Physiol. **107:** 306.
10. Sarnoff, S. J. & S. I. Yamada. 1959. Circulation Research. **7:** 325.
11. Eckenhoff, J. E. & J. H. Comroe, Jr. 1951. Proc. Soc. Exptl. Biol. Med. **76:** 725.
12. Coon, J. M. & S. Rothman. 1941. J. Pharmacol. Exptl. Therap. **73:** 1.
13. Hilton, S. M. 1954. J. Physiol. **123:** 289.
14. Johnston, L. M. 1942. Lancet. **2:** 742.
15. Gruzhit, C. C. 1957. Federation Proc. **16:** 303.
16. De Burgh Daly, M. & M. J. Scott. 1958. J. Physiol. **144:** 148–166; 1959. **145:** 440–446.

THE MECHANISM OF GANGLIONIC BLOCKADE PRODUCED BY NICOTINE*

Edward W. Pelikan

Department of Pharmacology and Experimental Therapeutics,
Boston University School of Medicine, Boston, Mass.

In textbooks that discuss the mechanism by which nicotine impairs ganglionic transmission, the mode of action generally accepted is that proposed by Paton and Perry (1953): "Nicotine appears to have a dual action, being initially a depolarizing, later a competitive blocking drug." In contrast, tetraethylammonium ion and hexamethonium ion were believed by Paton and Perry to impair ganglionic transmission by acting only as competitive drugs blocking acetylcholine released from presynaptic nerve terminals. The results of Lundberg and Thesleff (1953) were in agreement with those of Paton and Perry, in so far as the former found also that the ganglionic effects of nicotine occurred in two phases: an immediate depolarization of postsynaptic cells accompanied by impaired impulse transmission and a subsequent period of persistent impaired impulse transmission after repolarization was complete. Lundberg and Thesleff rejected the view that the second phase could result from a decrease of neurotransmitter from the presynaptic nerve endings, but they did not maintain that the second phase of ganglionic "block" by nicotine was the result of competition with the neurotransmitter. They adduced the evidence of Feldberg and Vartiainen (1935) that ganglionic block produced by nicotine was not accompanied by a decrease in acetylcholine content of the venous effluent of the blocked ganglion.

Experiments have led my colleagues and me to agree with Paton and Perry (1953) that hexamethonium produces ganglionic blockade by competing with the neurotransmitter for postsynaptic receptor sites. However it seems to us most probable that the impairment of ganglionic transmission produced by nicotine, which outlasts the period of stimulation or depolarization, is the result of an impaired release of neurotransmitter from presynaptic nerve terminals.

Paton and Perry (1953), Lundberg and Thesleff (1953), and we have studied transmission of impulses through the superior cervical ganglion of the cat. Paton and Perry and Lundberg and Thesleff studied essentially the demarcation potentials of the postganglionic nerve cell bodies and the action potential traffic in postganglionic nerve fibers. We have accepted the amplitude of contraction of the nictitating membrane evoked by ipsilateral preganglionic nerve stimulation as a measure of transmission through the ganglion. Our rejection of electrical recording techniques should, perhaps, be explained. Eccles (1935) first demonstrated that anoxia has trivial effects on the post-ganglionic action potentials; barring a slight increase in latency of response and a slight slowing of all waves, the action potentials continue unaltered and suffer a gradual decline in amplitude over a period of an hour or more. Marazzi (1939) has confirmed Eccles' observation and we, too, have made identical observations (Pelikan, unpublished observations). We have observed, however, that asphyxia of

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the animal or occlusion of the common carotid artery low in the neck leads, within a minute or two, to marked impairment or abolition of the response of the ipsilateral nictitating membrane to preganglionic stimulation; the degree of effect varies inversely with the frequency of stimulation. On the other hand, in our experience the occlusion of the external carotid artery well above the origin of the lingual artery (which decreases ipsilateral blood flow to the nictitating membrane, but leaves blood flow to the ganglion intact) does not lead to impaired responses of the membrane to preganglionic nerve stimulation. We have been forced to conclude that the electrical signs of ganglionic activity do not always reflect accurately the changes in the functional state of the ganglion.*

Ganglionic Block at High and Low Frequencies of Preganglionic Nerve Stimulation

Experiments were performed on 30 cats of both sexes anesthetized with sodium pentobarbital (35 mg./kg., intraperitoneally), supplemented by sodium phenobarbital given intravenously as needed. In every cat the trachea was cannulated, femoral arterial blood pressure was recorded kymographically using a mercury manometer, and a femoral vein was cannulated to permit intravenous injections. Stimuli were applied via shielded platinum electrodes to the right preganglionic nerve trunk low in the neck, and surgical manipulations in the region of the superior cervical ganglion were scrupulously avoided; square-wave stimuli of supramaximal voltage, 1 msec. duration, and of varying frequencies were obtained from a Grass Model S-4 stimulator. Isotonic contractions, with a 3.0-gm. load, of the ipsilateral nictitating membrane were recorded kymographically using an ink-writing muscle lever. The mid-line cervical incision was closed by suture after surgery was completed.

All injections of ganglionic blocking agents were made by rapid retrograde intra-arterial injection into the lingual artery in volumes of 0.5 ml. or less. Occlusion of the external carotid artery cephalad to the origin of the lingual artery during and shortly after the period of injection, in the manner of Trendelenburg (1956), ensured access of the bulk of the injected mass to the ganglion before drug reached the systemic circulation. Nicotine base, tetraethylammonium bromide, hexamethonium chloride, and morphine sulfate were dissolved in 0.9 per cent sodium chloride solution; the solutions were adjusted to *p*H 7.4 with sodium hydroxide and hydrochloric acid, and were administered at room temperature. Administration under the same conditions of solutions identical to these, but for the absence of drug, had no effect on the response of the membrane. Intra-arterial injections of drug solutions after occlusion of the external or common carotid artery just below the origin of the lingual artery affected the nictitating membrane response, if at all, only after a latency of 15 to 30 sec. Changes in systemic blood pressure following drug injections were poorly correlated in magnitude and time course with changes in response of the membrane. Usually more than one drug was administered during the course of an experiment.

Experiments were designed with the view that valid assay of drug antagonists involves comparison of the influence of various doses of antagonist on the effects of varying doses of the agonist. Acetylcholine, or other neurohumor, may be considered the agonist at the ganglionic synapse. Since the amount of acetylcholine released per nerve volley is independent of frequency of preganglionic nerve stimulation, at least at rates of from 2 to 20/sec. (Birks and MacIntosh, 1957), the amount of agonist released per unit time is proportional to stimulation frequency. Our experiments, therefore, involved analysis of the effects of nicotine and the drugs on the frequency response curve of the ganglionic transmission system.

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* This work has been reported previously only in abstract form (Pelikan and Garcia, 1959, 1960).

In our experiments, stimuli were applied at a low frequency for a period of 10 sec., after which a high stimulation frequency was applied for 10 sec.; the change in frequency was made abruptly by manual switching without the occurrence of a stimulus-free period. Such trains of stimuli were repeated at 1 to 3 min. intervals (after relaxation of the membrane had occurred) before and after all drug injections; a second dose of drug was not administered until the responses to stimuli had been constant for 4 to 6 or more minutes. The membrane response to the higher stimulation frequency was independent of whether or not a preceding period of low frequency stimulation had occurred. Qualitatively similar results were obtained with various pairs of frequencies, for example, 1.25/sec. and 20/sec., or 2/sec. and 20/sec., or 5/sec. and 20/sec. In our experiments we have routinely used frequencies of 1.25/sec. and 20/sec. The amplitude of membrane contraction at the two frequencies, respectively, were both measured from the base line present before low frequency stimulation was begun; the amplitude of contraction at the higher frequency was always larger than at the lower.

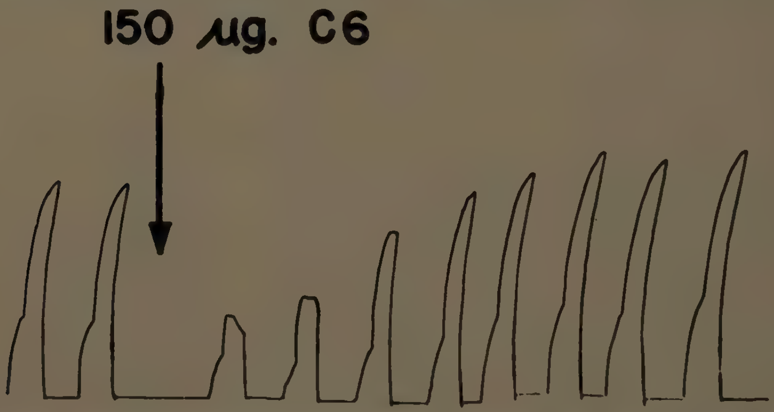

FIGURE 1. Response of cat nictitating membrane to low and high frequency stimulation.

Results typical of those seen after the administration of hexamethonium chloride (C6) (20 to 120 μg.) are displayed in FIGURE 1. After the administration of hexamethonium, which by itself never caused retraction of the membrane, ganglionic blockade was manifested by a marked decrease, relative to the control, of the response to the higher frequency of stimulation; the response to the lower frequency of stimulation was much less affected. In other terms, the ratio of the response to the higher frequency after drug administration

(HFR_a) to the low frequency response after the drug (LFR_a) was smaller than the ratio of the high frequency response before drug administration (HFR_b) to the low frequency response before the drug (LFR_b). The effect of hexamethonium was to *decrease* the ratio: $(HFR_a/LFR_a)/(HFR_b/LFR_b)$. During recovery from the effects of hexamethonium, the response to low stimulation frequencies returned to control values more quickly than did the high frequency response. The relatively greater sensitivity of the ganglion to hexamethonium at higher frequencies of preganglionic nerve stimulation was reported first by Paton and Zaimis (1951).

Remarkably different effects were seen after the administration of nicotine (FIGURE 2). With all doses of nicotine larger than 2 to 3 μg., injection was immediately followed by retraction of the nictitating membrane caused, presumably, by the depolarizing or stimulating effects of nicotine on the postsynaptic cell. With doses of nicotine from 20 to 80 μg., ganglionic blockade could be detected as an altered response to preganglionic nerve stimulation after the ganglionic stimulating effects of nicotine had passed off and the membrane had relaxed. In contrast to the effects seen after hexamethonium, the impaired ganglionic transmission induced by nicotine was manifested by a relatively greater decrease in low frequency response than in the response to the higher frequency of preganglionic nerve stimulation. With doses sufficient to affect markedly the response to low stimulation frequencies, the response to high stimulation frequencies was also affected somewhat, but the effect of nicotine was to *increase* the ratio $(HFR_a/LFR_a)/(HFR_b/LFR_b)$. With nicotine it was possible to abolish the low frequency response completely, without abolishing the high frequency response; that is, with nicotine it was possible to make the ratio $(HFR_a/LFR_a)/(HFR_b/LFR_b)$ infinitely large. Throughout the duration of action of a single dose of nicotine, that is, for as long as 20 to 25 min. in our experiments, the high frequency response was relatively less affected than was the response to the low stimulation frequency.

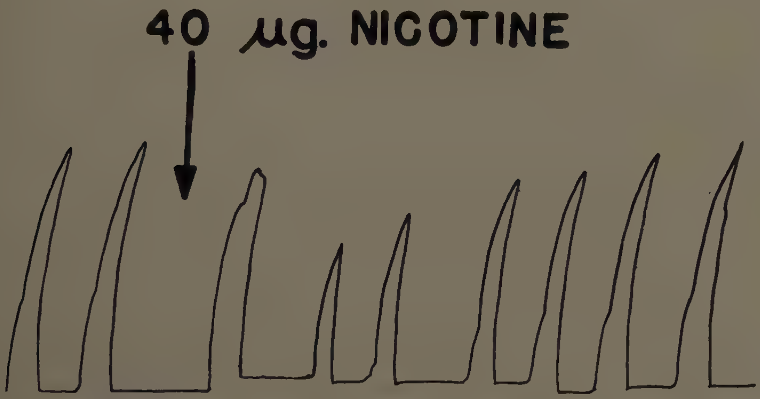

FIGURE 2. Responses of cat nictitating membrane to low and high frequency stimulation.

Neither with asphyxia, produced either by occlusion of the tracheal cannula or by occlusion of the carotid artery, nor with tetraethylammonium bromide (200 to 1000 μg.) alone was contraction of the membrane observed. But both asphyxia and tetraethylammonium bromide affected ganglionic transmission as did nicotine: the low frequency response was decreased relatively more than was the response to the higher frequency stimulation. Clark and Harvey (1959) have also reported that tetraethylammonium and hexamethonium differ in their effects on transmission through the superior cervical ganglion of the cat.

Feldberg and Vartiainen (1935) reported that nicotine did not impair release of acetylcholine from the superior cervical ganglion in which transmission had been impaired by nicotine. Feldberg and Vartiainen, however, give little quantitative data for their experiment and, in the specific experiment illustrated, they assayed for acetylcholine by a 1 × 1 assay using the blood pressure response of a cat with an intact autonomic nervous system as the criterion of drug effect: a somewhat hazardous way to assay a mixture of agents, both of which affect the autonomic nervous system of the cat. The response of our preparation to asphyxia suggested that asphyxia (and, by inference, nicotine and tetraethylammonium) affected the ganglion in a way that could be compensated for by increasing the frequency of preganglionic stimulation. Paton and Zaimis demonstrated that hexamethonium in effective doses did not decrease the amount of acetylcholine released from the perfused superior cervical ganglion. Knowing the difference in effects of nicotine, asphyxia, tetraethylammonium, and hexamethonium, and knowing that hexamethonium did not affect acetylcholine release from the ganglion, it was easy to hypothesize that nicotine, asphyxia, and tetraethylammonium blocked ganglionic transmission by preventing the release of neurotransmitter from presynaptic nerve terminals.

To test this hypothesis, we established a quantitative model of the ganglionic transmission process, using the following observations or assumptions:

1) The amount of acetylcholine (neurotransmitter) released in the ganglion per preganglionic volley, at least during a short period of stimulation, is independent of stimulation frequency (Birks and MacIntosh, 1957).

2) The amount of neurotransmitter released per unit time is a linear function of stimulation frequency. As did Rosenblueth (1932), we found the maximum response of the membrane to occur with preganglionic nerve stimulation at a rate of 20/sec. The amount of neurotransmitter released in 10 sec. with supramaximal stimulation at this rate was arbitrarily assigned a value of 100 units; the amount released at a frequency of stimulation of 1.25/sec. was 6.25 units.

3) The maximum contraction of which the nictitating membrane is capable when stimulated via pre- and postganglionic fibers is measured by the number of receptors on the postsynaptic cell body available for occupation by the neurotransmitter.

4) The fraction of available receptors occupied is related linearly to the ratio (neurotransmitter units released/total available receptors).

5) The fraction of the maximum contraction of the nictitating membrane produced by stimulation of the preganglionic nerve is measured by the logarithm of the fraction of the available postsynaptic receptors occupied by the neurotransmitter. The absolute amplitude of contraction of the membrane

depends, therefore, on both the fraction of available receptors occupied by neurotransmitter and the total number of receptors available for occupation.

6) Responses of the membrane of greater than 100 per cent are not possible under any circumstances. Calculated values larger than 100 per cent are arbitrarily reduced to that value.

With our mechanical recording system, the amplitude of membrane response was linearly related to the logarithm of the frequency of preganglionic nerve stimulation: in the normal ganglion a frequency of 1.25/sec. produced a response, on the average, 33 per cent of maximum, and a frequency of 20/sec. produced a maximum or 100 per cent response. On semilogarithmic graph paper, using "(neurotransmitter units released/total available receptors) times 100" as abscissal units on the logarithmic scale, and "per cent of maximum contraction" as ordinal units, we drew a straight line connecting the points (100, 100) and (6.25, 33). Using the postulates and the curve prepared as described, and by assigning arbitrary numerical values to the amount of neurotransmitter released per volley per unit time and the total number of postsynaptic receptors available for occupation by the neurotransmitter, it was possible to compute theoretical values for amplitude of contraction of the nictitating membrane with different frequencies of stimulation during normal ganglionic transmission and when ganglionic transmission was impaired by agents of two hypothetical types:

1) A type that could only decrease the amount of neurotransmitter released per volley from the nerve terminals of preganglionic fibers without altering the neurotransmitter or the postganglionic receptor mechanism in any way; and

2) A type that could only decrease the number of receptors on the postsynaptic cell body available to neurotransmitter released in normal quantities per volley from the presynaptic nerve endings.

Using computed values for response of the membrane to preganglionic stimulation, theoretical curves were plotted, each of which demonstrated the relationship between high frequency response and low frequency response before and after administration of agents of each of the two hypothetical types described above. The ordinal unit for such curves was the ratio described above: $(HFR_a/LFR_a)/(HFR_b/LFR_b)$. The abscissal unit was LFR_a/LFR_b, a measure of the effective dose of agent reaching the ganglion, expressed in terms of the effect of the dose on the low frequency response. The theoretical curves are presented as the curved lines of FIGURES 3, 4, and 5. The curve ascending to an ordinal value of infinity represents the relations among HFR and LFR before and after administration of an agent that only decreases the release of neurotransmitter from presynaptic terminals; the curve descending to an ordinal value of 0.33 represents these relationships before and after administration of an agent that impairs ganglionic transmission only by decreasing the number of postsynaptic receptors available to the neurotransmitter.

Experimental data from two representative experiments with nicotine are compared with the theoretical curves in FIGURE 3, and data from four representative experiments with hexamethonium are presented similarly in FIGURE 4. It should be pointed out that ordinal and abscissal units are dimensionless and permit uniform plotting of all membrane responses throughout the time course of action of all doses of an agent for all the experiments in which the action of that drug was studied. The data obtained after administration of nicotine fitted well the theoretical curve for an agent which impairs ganglionic transmission by only decreasing the amount of neurotransmitter released per nerve volley. Data obtained after administration of tetraethylammonium bromide or after asphyxiation of the cat also closely approximated this ascending curve. In contrast, the data for hexamethonium fitted the curve expected of an agent that blocks ganglionic transmission by only decreasing the number of post-synaptic receptors available for occupation by the neurotransmitter.

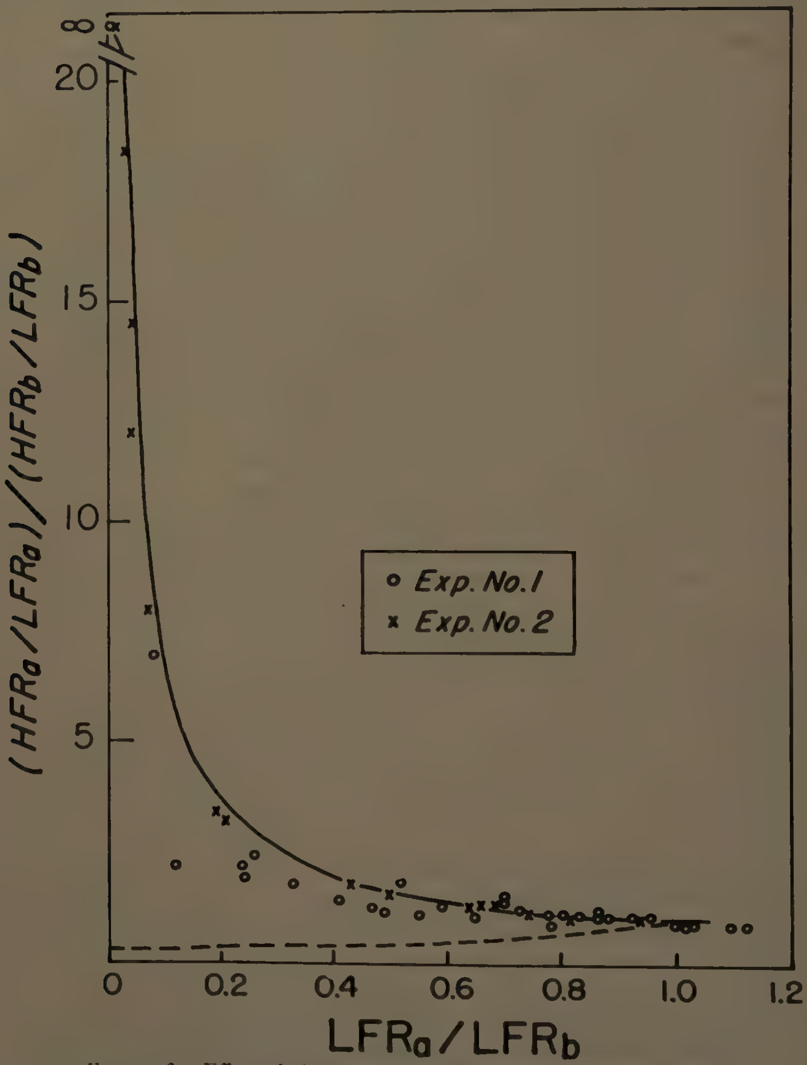

Figure 3. Effect of nicotine on ganglionic transmission in the cat.

Our data for hexamethonium are consistent with the mechanism of action of this agent proposed by Paton and Zaimis (1951); hexamethonium acts as a competitive antagonist to the ganglionic neurotransmitter.

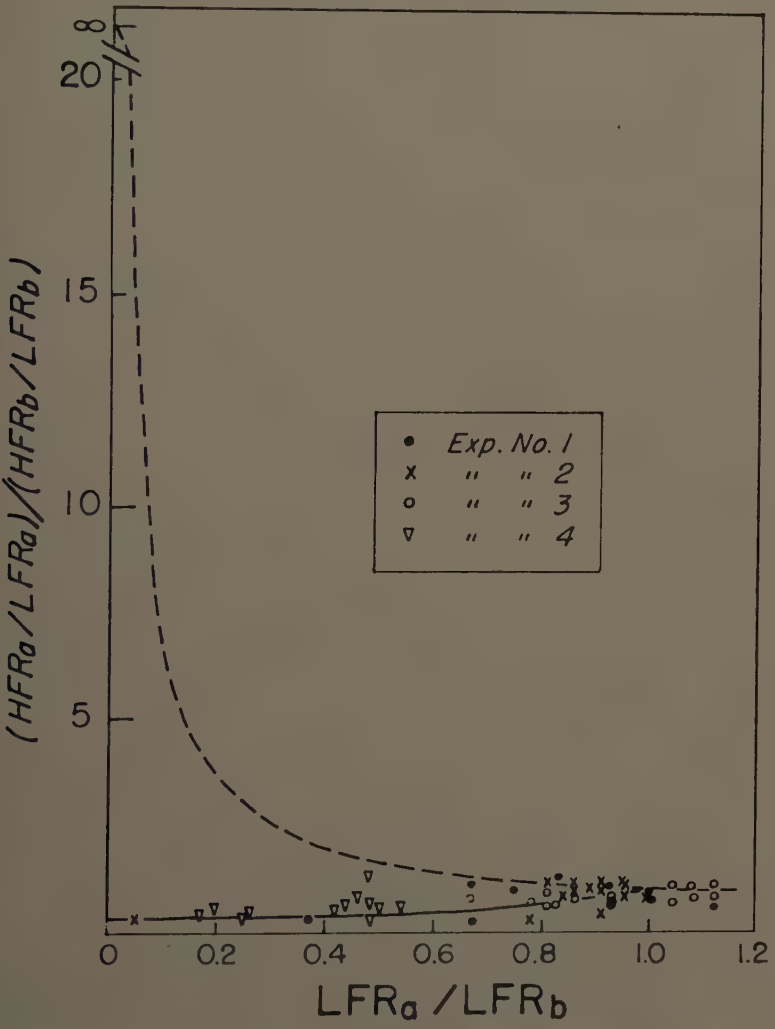

FIGURE 4. Effect of hexamethonium on ganglionic transmission in the cat.

We next sought confirmation of our theoretical formulations by studying the actions of morphine sulfate in impairing ganglionic transmission. Schaumann (1957) and Paton (1957) have demonstrated that morphine impairs the release of acetylcholine from isolated segments of guinea pig ileum. In our preparation, morphine sulfate (100–400 μg. intra-arterially) produced a reversible impairment of ganglionic transmission, without any preceding period of stimulation. As we had anticipated, the effects of morphine on the high and low frequency responses were consistent with the hypothesis that morphine acted only to decrease the amount of neurotransmitter released by each preganglionic nerve volley (FIGURE 5).

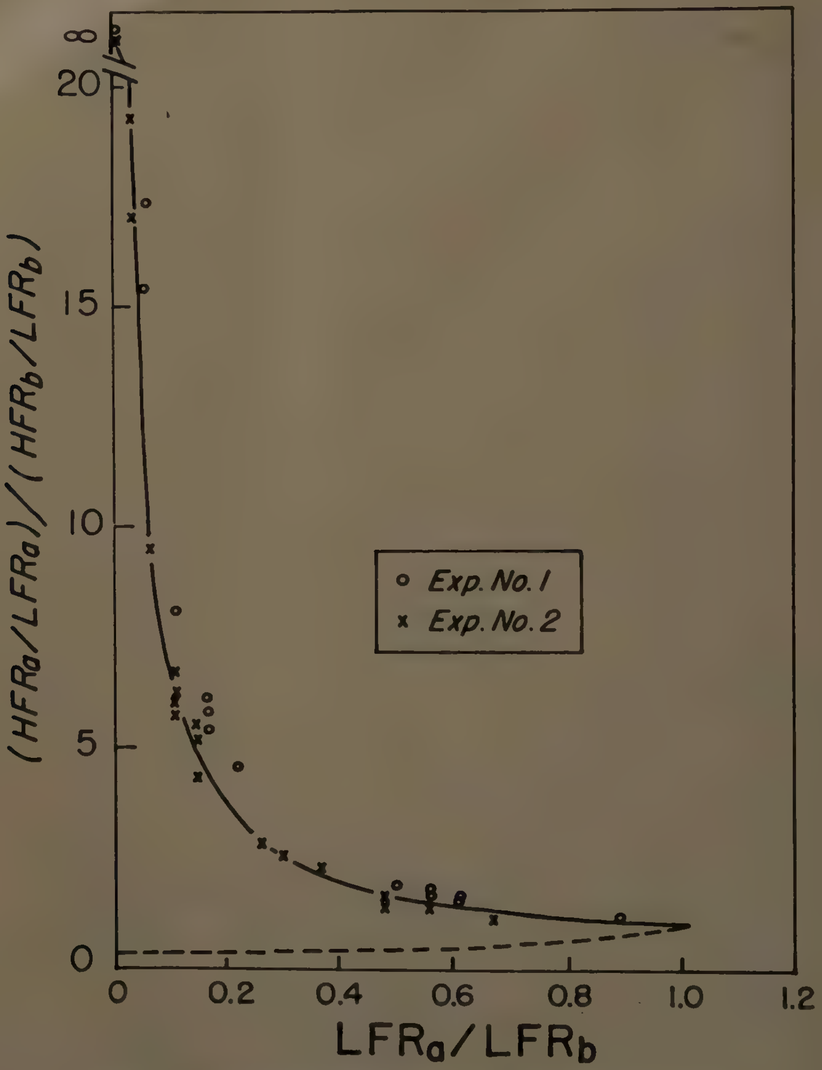

FIGURE 5. Effect of morphine on ganglionic transmission in the cat.

Experiments with Isolated Guinea Pig Ileum Segments

In order to test the hypothesis that nicotine might impair ganglionic transmission by impairing release of neurotransmitter, it was necessary to demonstrate that nicotine could impair release of acetylcholine from at least some organ and that methods could be developed that would permit detecting this effect. The necessity of measuring minute quantities of acetylcholine demanded the use of a biological assay procedure but, with the usual assay tissues, nicotine has effects qualitatively and quantitatively similar to those of acetylcholine.

Satisfactory assays of acetylcholine in the presence of nicotine were obtained using the isolated rat ileal segment as the assay tissue. Segments of ileum about 2.5 cm. long were removed from rats weighing about 150 gm. The segment was suspended in an oxygenated Tyrode's solution in a 10 ml. muscle bath maintained at a temperature of 37° C.; the solution contained glucose, magnesium, and calcium, and neostigmine bromide (1 μg./ml.). Unknown solutions and freshly prepared standard solutions of acetylcholine were added alternately to the muscle bath in constant volumes (0.5 ml.); contractions of the ileal segment were recorded kymographically using an isotonic, ink-writing muscle lever. Tension on the segments varied from 0.25 to 2.0 gm., but was held constant in any experiment.

All assays performed using the rat ileal preparation have been of a 2 × 2 design; assays have been performed after fitting a common slope to the dose-effect curves of the standard and unknown. All experiments (including those with effluent from the Kibjakov preparation, described below) that yielded sample volumes too small to permit a 2 × 2 assay design were rejected as incapable of yielding valid results. It was established in preliminary experiments that the rat ileal segment was more than 10,000 times as sensitive to the spasmogenic effects of acetylcholine as to the spasmogenic effects of nicotine; tetraethylammonium and hexamethonium in concentrations as high as 1 mg./ml. did not cause contraction of the rat ileum. Acetylcholine in concentrations as low as 1 to 10 ng. (0.001 to 0.01 μg.)/ml. could be assayed regularly with this preparation. Concentrations of nicotine, tetraethylammonium bromide, and hexamethonium chloride as high as 10 μg./ml., given before or with acetylcholine, had no effect on the response of the rat ileum to acetylcholine.

In more than 70 experiments, we studied the effects of nicotine, tetraethylammonium bromide, and hexamethonium chloride on the acetylcholine content of solutions in which guinea pig ileal segments had been incubated. Specifically, we determined the content of material spasmogenic for the rat ileum, assayed as acetylcholine. The guinea pig ileal segments were incubated at 37° C. in oxygenated Tyrode's solution containing glucose, magnesium, calcium, and neostigmine bromide (1 μg./ml.). The drug to be studied was added in predetermined concentrations to the incubation mixture; after 5 min. the medium was decanted to remove acetylcholine released during the ganglionic stimulating phase of nicotine's action and replaced by a like amount of fresh drug-containing solution. After one hour of incubation, the medium was removed and its spasmogen content determined, using the rat-ileum preparation. The effects of the spasmogen on the rat-ileum could be abolished by atropinization of the ileum or by boiling the sample.

As controls, some guinea pig ileum segments were boiled for 20 min. Such segments were then incubated, in the presence or absence of nicotine, tetraethylammonium bromide, or hexamethonium chloride; none of the media in which boiled ileal segments had been incubated contained spasmogen.

Nicotine (1 μg./ml.) in the incubation medium containing unboiled guinea pig ileal segments decreased the amount of spasmogen in the medium to 40 per cent, on an average, of the amount contained in medium from control flasks containing unboiled segments without nicotine. No spasmogen was found in the medium that had contained unboiled ileal segments and nicotine (10 μg./ml.). We concluded that nicotine can, in fact, cause a decrease in the amount of acetylcholine released from a tissue which contains ganglia. There is no reason to believe that the effect of nicotine on the spasmogen content of the medium was the result of an impaired synthesis of acetylcholine. Fahmy *et al.* (1954) have shown that concentrations of nicotine even larger than those used by Bain (1950) were required to decrease the cholinacetylase activity of rat brain by 50 per cent. Bain (1950) demonstrated that nicotine had only an inhibitory effect on rat brain cholinesterase in concentrations about 1000 times as great as we have used.

In contrast to the results obtained with nicotine, neither hexamethonium chloride nor tetraethylammonium bromide, both in concentrations as high as 1 mg./ml., had any effect on the spasmogen content of the incubation medium for the guinea pig ileal segments. We anticipated the lack of effect of hexamethonium on the basis of the results reported by Paton and Zaimis (1951); we had not anticipated the lack of effect of tetraethylammonium since it affected the nictitating membrane response to preganglionic stimulation in exactly the way that nicotine had. Nor are we able to account for the apparent disparity in the results of our *in vivo* and *in vitro* experiments with tetraethylammonium. With concentrations of the agent as high as 1 mmole, Kumagai *et al.* (1954) found neither inhibitory nor facilitatory effects of tetraethylammonium (or of hexamethonium) on the cholinacetylase activity of rat brain. Tetraethylammonium ion has been shown to have facilitatory and inhibitory effects on cholinesterases under different experimental conditions (Kalow, 1960; Erdös *et al.*, 1958; Kensler and Elsner, 1951). Stovner (1958), however, has concluded from the results of his studies of the actions of tetraethylammonium at the skeletal neuromuscular junction that the agent causes a release of acetylcholine from motor nerve terminals. The complexity of organization of the intestinal strip makes it impossible to interpret our results with tetraethylammonium ions *in vitro*.

Experiments with the Kibjakov Preparation

With the evidence at hand that nicotine could decrease the output of acetylcholine from an organ, and that the decrease could be detected and measured, we attempted to assess the effects of nicotine on the output of acetylcholine from the perfused superior cervical ganglion of the cat. Paton and Zaimis (1951) have already reported that in this preparation hexamethonium can produce ganglionic blockade without impairing acetylcholine release.

The right superior cervical ganglion of 23 barbitalized tracheotomized cats was perfused essentially in the manner described by Kibjakov (1933). Oxygenated glucose-containing Ringer's solution, containing magnesium, calcium, and neostigmine bromide (1 μg./ml.), was perfused cephalad into the common carotid artery under a pressure head of 80 to 120 mm. Hg, using a constant volume pulsatile-flow perfusion pump. Perfusion pressure and systemic arterial blood pressure were monitored continuously by means of mercury manometers. Before entering the artery the fluid was warmed in the manner described by Perry (1953). All visible arterial branches above the site of entrance of the cannula were ligated and cut, including the external carotid artery, but excluding the lingual artery (through which retrograde intra-arterial injections were made as described above) and excluding the branch or branches passing to the ganglion. In none of our experiments could the internal jugular vein be cannulated, even with a 25-gauge hypodermic needle; hence, venous effluent was collected via a cannula of less than 0.25 ml. volume placed in a branch of the posterior facial vein. Visible venous branches draining areas other than the ganglion were ligated and cut. Venous effluent was collected and measured continuously, but over time periods held constant in length during any given experiment. Venous outflow in different experiments ranged, in the absence of nicotine, between 0.5 and 2.5 ml./min.

Stimuli were applied to the ipsilateral preganglionic trunk at a frequency of 20/sec. and the contractions of the ipsilateral nictitating membrane were recorded in the fashion described above. Nicotine solutions identical to those previously used were injected to the ganglion via the lingual artery; nicotine doses of 100 μg. were used.

The spasmogen content of the effluent was assayed against an acetylcholine standard using the rat ileum preparation. Results were expressed as output of spasmogen, as acetylcholine, in ng./min. Only the results of 2 × 2 assays were considered valid. Samples of effluent were stored on ice for no more than two hours before being assayed. The spasmogenic activity of the effluent for the rat ileum could be abolished by atropinization of the ileum or by boiling the sample.

Three experiments were technically adequate to permit study of the effects of nicotine on the output of acetylcholine from the ganglion. In all three, in contrast to the report of Feldberg and Vartiainen (1934), injection of nicotine in a dose sufficient to prevent the response of the nictitating membrane to preganglionic nerve stimulation was followed by a decrease in the acetylcholine content of effluent from the ganglion. Results of two of the experiments are portrayed in FIGURES 6 and 7.

In FIGURES 6 and 7 open circles indicate the quantities of acetylcholine in samples of effluent obtained in the absence of preganglionic nerve stimulation, closed circles correspond to samples obtained during periods of preganglionic nerve stimulation. The length of the horizontal lines associated with the circles indicate the periods during which the samples were collected and the duration of preganglionic stimulation when it was applied. In FIGURE 6, the horizontal line also represents the geometric mean acetylcholine content of replicate assays on single effluent samples.

The figures demonstrate, first, that unlike the data reported by MacIntosh (1938), we have not invariably found the output of acetylcholine from the unstimulated ganglion to be zero, or negligible, nor have we found that the acetylcholine output from the stimulated ganglion was invariably greater than the output from the unstimulated ganglion. Second, the figures demonstrate no increase above control levels of acetylcholine content of samples of effluent obtained immediately after injection of nicotine; that is, there was no apparent change in acetylcholine output that could be attributed to a release of acetylcholine by nicotine or to a spurious assay resulting from the presence of nicotine in the effluent.

It can be seen in FIGURES 6 and 7, however, that injection of nicotine into the Kibjakov preparation was followed by the usual local circulatory changes (see below), by abolition of the response of the nictitating membrane to preganglionic nerve stimulation, and by a simultaneous abrupt decrease in the amount of acetylcholine released into the effluent from the ganglion. The paralysis of the nictitating membrane (to which the blood supply had been drastically reduced) occurred abruptly after the administration of nicotine and was usually irreversible. In one experiment however (not pictured here) acetylcholine output returned to control levels following the decreased output produced by nicotine.

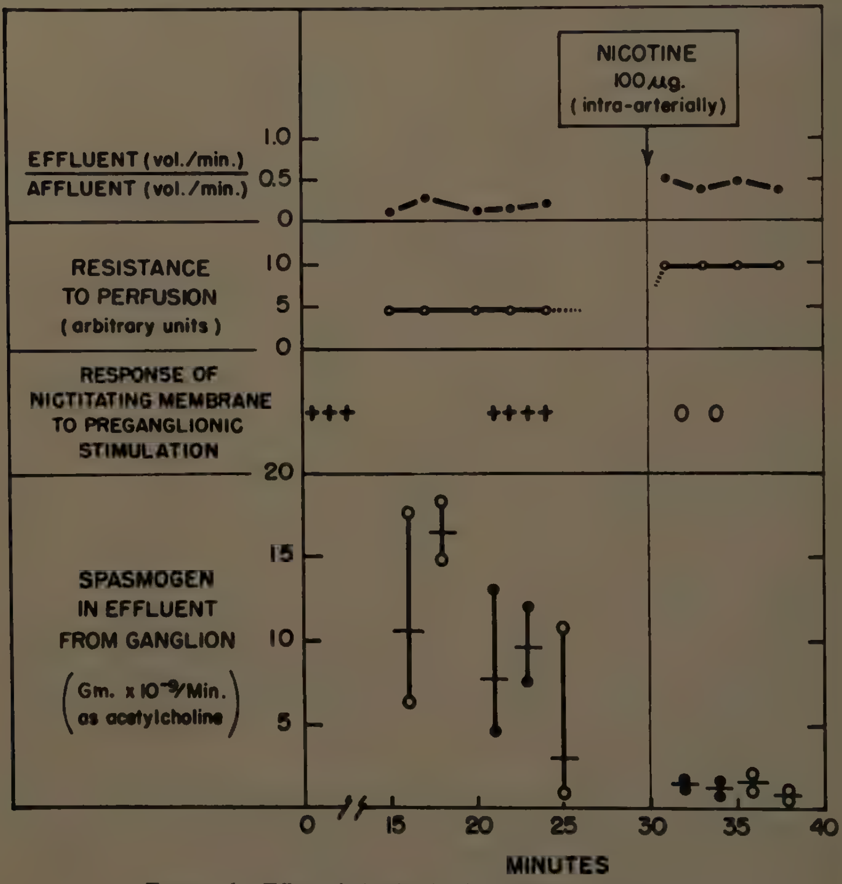

FIGURE 6. Effect of nicotine in the Kibjakov preparation.

Our results are consistent with the hypothesis that the impairment of ganglionic transmission produced by nicotine that follows the stimulant effect of

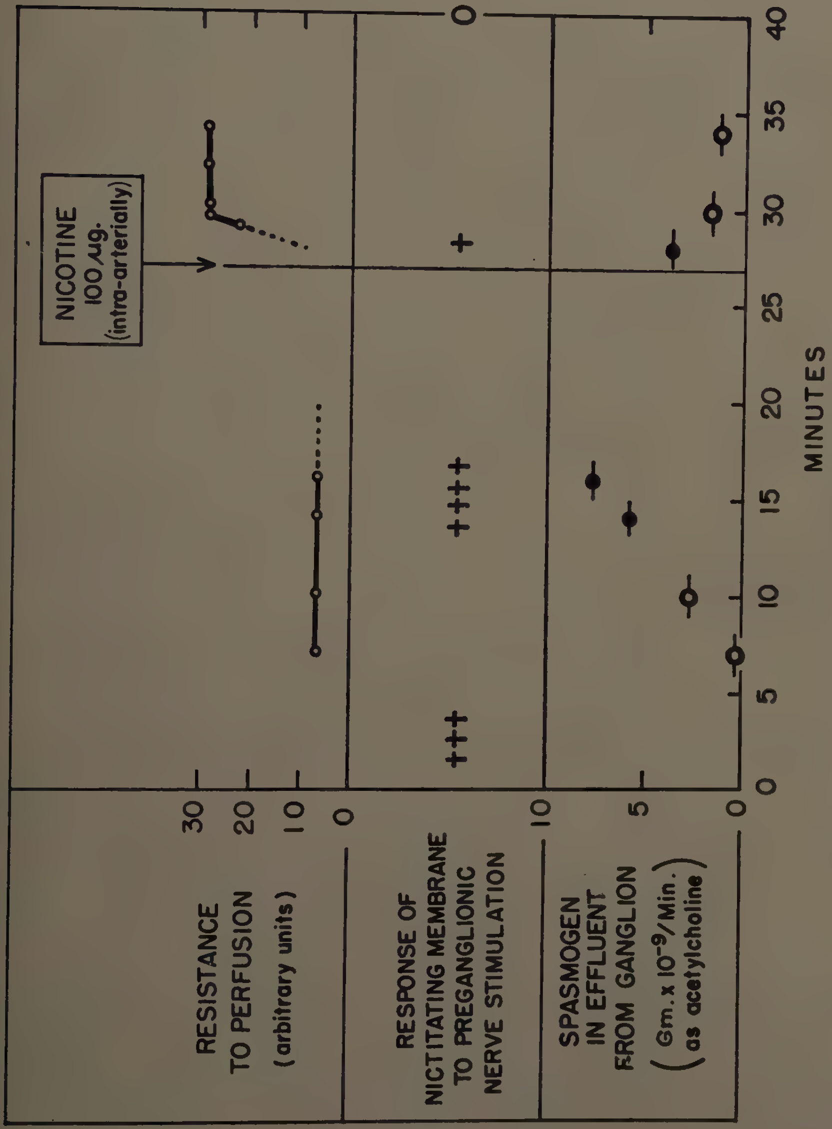

FIGURE 7. Effect of nicotine in the Kibjakov preparation.

nicotine on the ganglion, is a result of an action of nicotine to decrease the amount of neurotransmitter released per nerve volley from the preganglionic nerve terminals, rather than the result of an effect of nicotine to make post-synaptic cell receptors unavailable to the neurotransmitter.

In contrast to the results implicit in most of the reports in the literature, we were unable surgically to isolate completely the circulation of the ganglion from the systemic circulation while the animal was alive. FIGURE 8 displays the results of an experiment performed specifically to demonstrate this. As can be seen from the figure, immediately after beginning perfusion the hemoglobin concentration of the effluent was greater than that of the systemic venous blood (hemoglobin was measured photocolorimetrically after hemolysis with saponin, and is expressed as a percentage of the initial hemoglobin concentration of systemic venous blood determined on replicate samples). During this period, the quantity of effluent recovered was less than half of the volume infused; Brücke (1935) too, failed to recover the perfusate quantitatively. After ligation of the contralateral common carotid artery and both jugular veins, the hemoglobin concentration of the effluent became less than that of the systemic venous blood, but still more fluid was infused than was recovered. The con-

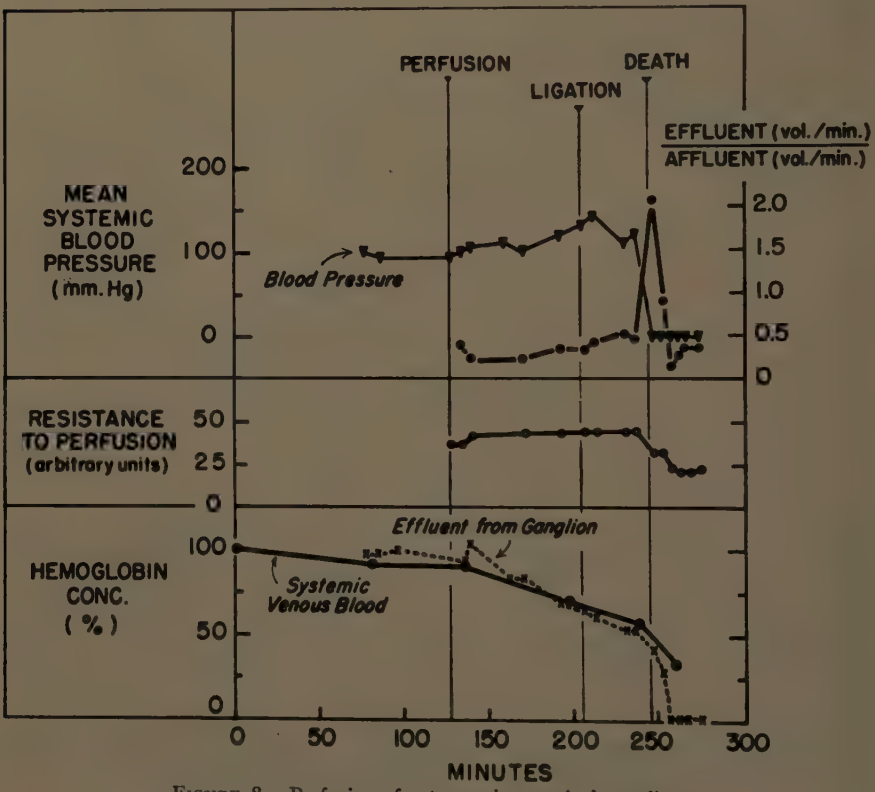

FIGURE 8. Perfusion of cat superior cervical ganglion.

tinuous and progressive decrease in the systemic venous blood hemoglobin concentration throughout the course of the experiment probably was caused by continuous loss of perfusate into the systemic circulation. Only after death of the animal by asphyxiation did the effluent become free of hemoglobin. After a transient increase in the amount of effluent relative to the amount infused at the time of asphyxia, more fluid again was infused than was recovered. Death of the animal resulted in a significant decrease in resistance to perfusion ([outflow in ml./min.]/[perfusion pressure in mm. Hg]) in the perfused area.

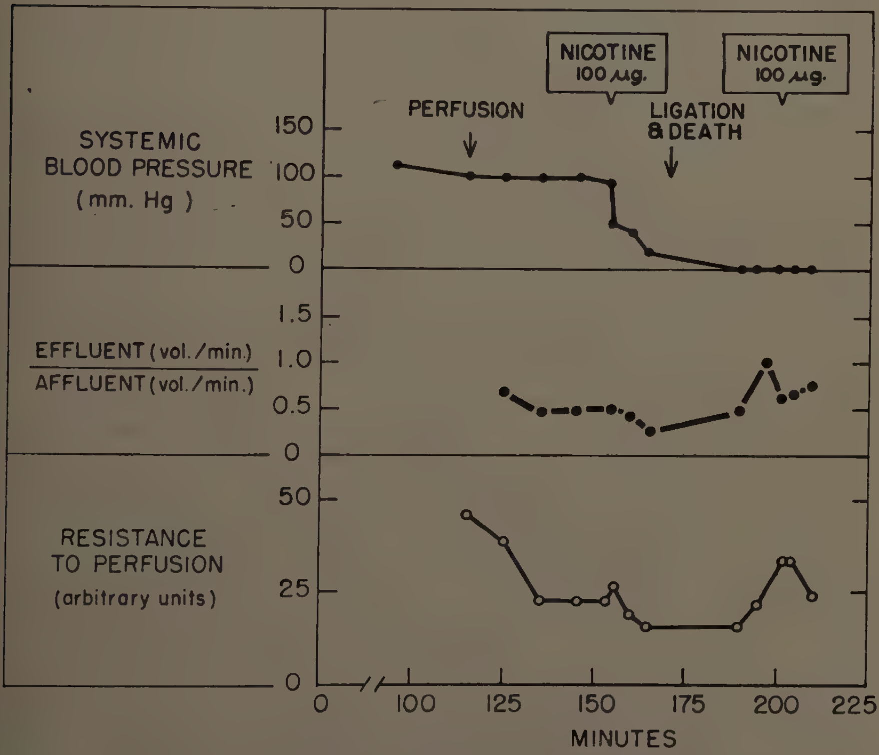

Figure 9. Perfusion of cat superior cervical ganglion.

As a consequence of experiments such as those documented by figure 8, studies on the effects of nicotine on acetylcholine release were carried out only in dead cats in which the contralateral major cervical vessels had been ligated.

In contrast to the results implicit in most reports in the literature concerning the Kibjakov preparation, we observed that preganglionic nerve stimulation, and the injection of nicotine, altered the circulatory dynamics of the perfused tissues. This effect of nicotine is pictured in figure 9. Administration of nicotine, before or after ligation of the contralateral cervical vessels and death of the animal, usually resulted in increased resistance to perfusion and an absolute decrease in the volume outflow per minute from the venous cannula. Frequently, but not in the experiment pictured in figure 9, following the injection of nicotine there was an increase in the volume flow of effluent relative to the volume flow of affluent, and the previously clear effluent became blood-tinged. These same effects were observed, albeit less consistently, after preganglionic nerve stimulation. Similar results were obtained after preganglionic nerve stimulation or injections of epinephrine in the isolated perfused rabbit head preparation by Wierzuchowski (1938) and Regnier (1926). Communication of the perfused vasculature and the systemic circulation is illustrated in FIGURE 9 by the marked decrease in systemic blood pressure produced by injection of nicotine in the living animal.

The effects of nicotine and preganglionic nerve stimulation on the circulatory dynamics of the area perfused in the Kibjakov preparation seem best explained as a net decrease in blood flow in the perfused area caused by vasoconstriction induced by stimulation of vasomotor nerve cell bodies in the ganglion. The transient appearance of blood in the effluent and the usual increase in *relative* volume flow of effluent following such stimulation suggests that, with restriction of blood flow to areas previously easily perfused, there is a simultaneous change in venous outflow: after nicotine injection or nerve stimulation some areas contribute to the total venous return that probably did not contribute before.

Nothing quantitative is known about the partition of affluent volumes among various tissues to which the perfusate has access, nor about the relative importance of various sources of venous effluent or the neurotransmitter output from areas other than the ganglion perfused. Hence it is not feasible to evaluate, quantitatively, the relationship of these circulatory changes to the observed effects of nicotine on the acetylcholine content of the effluent. However, in one cat killed by asphyxiation before perfusion was begun and upon which a sham experiment was performed, no material spasmogenic to the rat ileum was found in any effluent sample. The amount of spasmogen that could have been present in any sample in this experiment was equivalent to an output as acetylcholine of less than 0.1 ng./min., less than one fiftieth of the output usually obtained from a preparation containing a functional ganglion.

Summary and Conclusions

Before the publication of Clark's monograph (Clark, 1937), it was customary to attribute the effects of many drugs to actions on nerve endings. After Clark's publication, attention was focused on postsynaptic cell receptors as a site of drug action. Within only the last few years has evidence again been interpreted as indicating that presynaptic mechanisms may be modified by drugs that affect synaptic transmission. However, the presynaptic nerve terminals in ganglia have not previously been suggested as the locus at which nicotine acts to block ganglionic transmission at a time beyond the period of ganglionic stimulation. The fit of the experimental data to the theoretical curves, the results of our *in vitro* experiments, and the consistency of these results with those obtained using the Kibjakov preparation have led us to the following conclusions:

(1) In even the lowest doses that block ganglionic transmission in the superior cervical ganglion of the cat, nicotine, tetraethylammonium bromide,

and morphine impair impulse transmission by a mechanism fundamentally different from that by which hexamethonium chloride affects transmission.

(2) Hexamethonium probably blocks ganglionic transmission by competing with acetylcholine for postsynaptic receptor sites in the manner suggested originally by Paton and Zaimis.

(3) In contrast, the ganglionic blockade produced by nicotine, which outlasts the period of depolarization of the postsynaptic cells, is probably the result of an impaired release of acetylcholine from presynaptic nerve terminals, rather than the result of a competition for postsynaptic receptor sites between nicotine and acetylcholine.

Acknowledgment

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References

Bain, J. A. 1950. Am. J. Physiol. **160:** 187.
Birks, R. I. & F. C. MacIntosh. 1957. Brit. Med. Bull. **13:** 157.
Brücke, F. T. 1935. Arch. exptl. Pathol. Pharmakol. Naunyn-Schmiedeberg's. **177:** 532.
Clark, A. J. 1937. General Pharmacology. *In* Heffter's Handbuch der experimentellen Pharmakologie, Erganzbd. IV. Berlin, Germany.
Clark, W. G. & S. C. Harvey. 1959. Federation Proc. **18:** 377.
Eccles, J. C. 1935. J. Physiol. **85:** 179.
Erdös, E. G., F. F. Foldes, E. K. Zsigmond, N. Baart & J. A. Zwartz. 1958. Science. **128:** 92.
Fahmy, A. R., B. E. Ryman & E. O'F. Walsh. 1954. J. Pharm. and Pharmacol. **6:** 607.
Feldberg, W. & A. Vartiainen. 1935. J. Physiol. **83:** 103.
Kalow, W. 1960. Federation Proc. **19:** 276.
Kensler, C. J. & R. W. Elsner. 1951. J. Pharmacol. Exptl. Therap. **102:** 196.
Kibjakov, A. W. 1933. Arch. ges. Physiol. Pflüger's. **232:** 432.
Kumagai, H., S. Ebashi & F. Takeda. 1954. Japan. J. Pharmacol. **4:** 24.
Lundberg, A. & S. Thesleff. 1953. Acta Physiol. Scand. **28:** 218.
MacIntosh, F. C. 1938. J. Physiol. **94:** 155.
Marazzi, A. S. 1939. J. Pharmacol. Exptl. Therap. **65:** 395.
Paton, W. D. M. 1957. Brit. J. Pharmacol. **12:** 119.
Paton, W. D. M. & W. L. M. Perry. 1953. J. Physiol. **119:** 43.
Paton, W. D. M. & E. Zaimis. 1951. Brit. J. Pharmacol. **6:** 155.
Pelikan, E. W. & G. C. Garcia. 1959. Federation Proc. **18:** 432.
Pelikan, E. W. & G. C. Garcia. 1960. Federation Proc. **19:** 284.
Perry, W. L. M. 1953. J. Physiol. **119:** 439.
Regnier, P. 1926. Arch. intern. pharmacodynamie. **31:** 303.
Rosenblueth, A. 1932. Am. J. Physiol. **100:** 443.
Schaumann, O. 1957. Brit. J. Pharmacol. **12:** 115.
Stovner, J. 1958. Acta Pharmacol. Toxicol. **14:** 317.
Trendelenburg, U. 1956. J. Physiol. **135:** 259.
Wierzuchowski, M. 1938. Arch. intern. pharmacodynamie. **58:** 47.

ACTION OF NICOTINE ON THE HEART

J. Harold Burn
Balliol College, University of Oxford, Oxford, England

Indirect Effects of Nicotine

In describing the action of nicotine on the heart, certain indirect actions will be considered first. Nicotine stimulates sympathetic ganglia and therefore raises the blood pressure. Nicotine also increases the discharge of epinephrine from the adrenal medulla, and epinephrine dilates the coronary vessels. The combination of these two effects must improve the coronary flow.

Nicotine also stimulates the cells of the supraoptic nucleus, from which impulses pass to the posterior lobe of the pituitary and cause a discharge of vasopressin. Vasopressin causes a reduction of coronary flow. However, the amounts of vasopressin detected in the venous blood of man after smoking cigarettes have been too small to cause a significant reduction, although these amounts are sufficiently large to have a pronounced antidiuretic effect on the kidney (Burn *et al.*, 1945).

Direct Effects of Nicotine

The fibers of the vagus nerve that go to the heart are preganglionic fibers. They terminate around ganglia in the atria of the heart. These ganglia give off short fibers that are postganglionic; at the terminations of these fibers acetylcholine is liberated. Nicotine stimulates these vagal ganglia and, as a result, produces an inhibitory effect, retarding the heart rate. This inhibitory effect is seen in the isolated heart or in the isolated atria after removal from the body, when the contractions of the heart or atria are maintained by perfusion with or immersion in some form of Ringer's solution. The inhibitory effect is abolished by atropine.

There is, however, another direct effect, described by Dixon (1924) in his article in Heffter's *Handbuch.* Even in the absence of atropine, the inhibitory effect of nicotine is followed by an augmentor effect, the rate and force of the beat being increased. Dixon supposed that all effects of nicotine were due to ganglionic stimulation, and he believed that the augmentation must mean that sympathetic ganglia were also present within the tissues of the heart. Anatomically, the sympathetic ganglia giving rise to the accelerator nerves to the heart are the stellate ganglia, and these lie outside the heart at the head end of the thoracic sympathetic chain. There is in fact no evidence to support Dixon's explanation.

Acetylcholine Releases Epinephrine

Light was thrown on this augmentor action of nicotine by the work of Hoffman *et al.* (1945). Actually, the observations of these investigators were concerned almost entirely with the augmentor action of acetylcholine and not with that of nicotine, although they mention nicotine in their paper. As we have seen in the case of nicotine, these workers showed that acetylcholine also had two effects in the heart. The first was its inhibitory effect, which was abolished by atropine. The second was an augmentor effect seen in the presence of atropine. This augmentor effect is, in all probability, identical with the augmentor effect of nicotine.

The important contribution of these workers was that they showed that, when acetylcholine caused an augmentation of the rate and force of the beat of the isolated perfused heart, a substance was liberated in the perfusate leaving the heart that resembled epinephrine in its action. The perfusate, when tested on the frog's heart, increased the force of contractions; when tested on the hen's rectal cecum, it caused inhibition; when tested on the rabbit's small intestine, it also caused inhibition. The perfusate coming from the heart when it was not stimulated by acetylcholine had none of these effects. It therefore appeared that the augmentor effect of acetylcholine was due to the release of a substance like epinephrine or norepinephrine from stores present in the heart.

Nicotine on Atria

Direct observations of the action of nicotine on atria from the rabbit's heart suspended in a bath were obtained by Kottegoda (1953), who observed an initial inhibition followed by stimulation of rate and force of the contractions and studied the stimulation in the presence of atropine when the inhibition was excluded. Kottegoda showed that the augmentation did not occur in the presence of hexamethonium, a substance that blocks the action of nicotine on ganglia. This finding recalled Dixon's idea that there might be sympathetic ganglia within the atrial tissue on which nicotine exerted its action.

Stores of Norepinephrine

Norepinephrine was shown to be present in the heart by Goodall (1951), together with much smaller amounts of epinephrine. That is to say, when the heart was extracted, norepinephrine could be found in the extract.

In 1953 the properties of the alkaloid reserpine were described, and the first investigations were directed chiefly to its effects on the brain. The important observation was made by Holzbauer and Vogt (1956) that, when reserpine was administered to cats, the amount of norepinephrine present in the brain, particularly in the hypothalamus, was greatly reduced. Then a paper was published by Bertler *et al.* (1956) showing that, when reserpine was given by intravenous injection to a rabbit, the norepinephrine present in the heart also was greatly reduced. The authors found in the hearts of normal rabbits a mean amount of norepinephrine equivalent to 1.57 μg./gm. In the hearts of rabbits excised 16 hours after treatment with reserpine, the mean amount was 0.03 μg./gm.

Nicotine on Atria from Reserpine-Treated Rabbits

We were interested, therefore, to test the effect of nicotine on the atria freed from norepinephrine by the action of reserpine. We found that, in the presence of atropine, nicotine had no action of any kind on the rate or on the force of the beat. This finding made it certain that the augmenting action of nicotine was due to the release of norepinephrine from the stores in the heart (Burn and Rand, 1958).

When making these observations, we observed that there was a difference between the rate of contraction of the atria taken from normal rabbits and the rate of those from rabbits treated with reserpine. Whereas in a bath at 30° C. the atria from normal rabbits contracted at 146/min., the atria from those treated with reserpine contracted at 112/min. These were the mean figures for groups of 10 atria. The difference was highly significant ($P = 0.001$), as there was little overlap between the groups (TABLE 1). The difference suggested that in normal animals there was a small discharge from the store of norepinephrine that increased the activity of the pacemaker.

Presumably, the same situation exists in the human heart. The extent of the discharge of norepinephrine from the store is likely to vary in different subjects and may account for the difference in the rate of the pacemaker in these subjects.

TABLE 1

SPONTANEOUS RATE OF DIFFERENT ATRIAL PREPARATIONS AT 30° C. (BEATS PER MINUTE)

	Controls	Atria from rabbits treated with reserpine
	132	128
	132	136
	164	116
	168	112
	170	100
	144	92
	136	104
	152	108
	168	138
		100
		104
Mean	146	112

Uptake of Norepinephrine by Atria

When the effect of nicotine on normal atria has been observed, using a moderate concentration (this would be 2×10^{-6} gm./ml. nicotine base, or 6 μg./ml. nicotine acid tartrate), the effect of nicotine can be increased by adding norepinephrine to the bath in a concentration of 1 μg./ml. and leaving it for 30 min. If at the end of this time the norepinephrine is washed out and the bath fluid is changed a second time, the addition of the same concentration of nicotine causes a greater increase in rate than before. This indicates that norepinephrine is taken up by the atria from the fluid in the bath. A similar observation was made by Gillespie and Mackenna (1959) on the isolated rabbit colon.

Effect of Smoking on Heart Rate

These observations indicate that the effect of smoking in increasing the heart rate is not due to stimulation of the stellate ganglia, although that may contribute, but to the release of norepinephrine from the store in the heart. Repeated smoking may therefore be expected to produce tachycardia in sensitive subjects, and to precipitate those arrhythmias that are aggravated by norepinephrine.

Location of the Store

The location of the store is still uncertain, but Goodall (1951) showed that, after removing the stellate ganglia in sheep and allowing time for degeneration, the amount of norepinephrine that could be extracted from the heart was then very small. This indicated that the store was near the terminations of the sympathetic fibers.

Much earlier, Kohn (1903) described the presence of chromaffin cells, which contain epinephrine, in sympathetic ganglia and sympathetic trunks. These he called paraganglia, because embryologically they had the same origin as the sympathetic ganglia. Later, Wiesel (1906) described chromaffin tissue in the human heart, locating what he called the paraganglion cardiacum near the origin of the left coronary artery. Trinci (1907) showed that chromaffin tissue was present in the hearts of guinea pigs and cats. Still later Busacchi (1912) described chromaffin tissue in the human heart in the neighborhood of both coronary arteries, especially the left.

Thus it is clear that nicotine may liberate norepinephrine or epinephrine from chromaffin tissue in the heart, and in this way produce acceleration.

Such an explanation seems more likely than one stating that there are sympathetic ganglion cells, but it is an explanation that is only provisional.

References

BERTLER, A., A. CARLSSON & E. ROSENGREN. 1956. Release by reserpine of catecholamines from rabbits' hearts. Naturwiss. **43:** 521.

BURN, J. H. & M. J. RAND. 1958. The action of nicotine on the heart. Brit. Med. J. **i:** 137.

BURN, J. H., L. H. TRUELOVE & I. H. BURN. 1945. The antidiuretic action of nicotine and of smoking. Brit. Med. J. **i:** 403.

BUSACCHI, P. 1912. I corpi cromaffini del cuore umano. Arch. ital. Anat. e embriol. **11:** 352.

DIXON, W. E. 1924. Nicotin, Coniin, Piperidin, Lupetidin, Cytisin, Lobelin, Spartein, Gelsemin. *In* Heffter's Handbuch der experimentellen Pharmakologie. Part 2. **2:** 656.

GILLESPIE, J. S. & B. R. MACKENNA. 1959. The effect of reserpine on the response of the rabbit ileum and colon to stimulation of the extrinsic nerves *in vitro*. J. Physiol. **147:** 31P.

GOODALL, McC. 1951. Studies on noradrenaline and adrenaline in mammalian heart. Acta Physiol. Scand. **24:** Suppl. 85.

HOFFMAN, F., E. J. HOFFMAN, S. MIDDLETON & J. TALESNIK. 1945. The stimulating effect of acetylcholine on the mammalian heart and the liberation of an epinephrine-like substance by the isolated heart. Am. J. Physiol. **144:** 189.

HOLZBAUER, M. & M. VOGT. 1956. Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. J. Neurochem. **1:** 8.

KOHN, A. 1903. Die Paraganglien. Arch. milkroskop Anat. u. Entwicklungsmech. **62:** 263.

KOTTEGODA, S. R. 1953. Stimulation of isolated rabbit auricles by substances which stimulate ganglia. Brit. J. Pharmacol. **8:** 83.

TRINCI, G. 1907. Cellule cromaffini e "Mastzellen" nella regione cardiaca dei Mammiferi. R. Accad. delle Scienze di Bologna. **4:** Ser. 6.

WIESEL, J. 1906. Über Erkrankungen der Koronararterien im Verlaufe akuter infektionstankheiten. Wien. Klin. Wochenschr. **24:** 723.

THE EFFECT OF NICOTINE AND SMOKING ON THE SECRETION OF EPINEPHRINE*

Daniel T. Watts

Department of Pharmacology, West Virginia University Medical Center, Morgantown, W. Va.

As early as 1912 Dale and Laidlaw[1] found that the pressor response of the cat to nicotine was due at least partially to the liberation of epinephrine by the adrenal glands. Van Slyke and Larson[2] have shown that the pressor response to nicotine in dogs is greatly reduced following adrenalectomy. They indicate that release of epinephrine from the adrenal medulla is an important factor in this pressor response to epinephrine. More recently, with the development of fluorimetric and bioassay methods for the quantitative estimation of epinephrine and norepinephrine, the liberation of these substances into the adrenal vein blood has been observed following the administration of nicotine. De Schaepdryver[3] has shown that the administration of 0.1 mg./kg. nicotine increased the norepinephrine secretion of the adrenal gland of dogs from a control output of 7 ng.†/kg./min. to 555 ng./kg./min. The epinephrine output was increased from 12 to 1327 ng./kg./min. The epinephrine represented 63 per cent of the total catechols before nicotine and 71 per cent following administration of the compound. Other investigators[4,5] have observed comparable increases in the release of epinephrine from the adrenal glands of dogs following the intravenous administration of nicotine, epinephrine accounting for 80 to 90 per cent of the catecholamines of the adrenal vein blood.

Peripheral Blood Epinephrine Levels in Dogs After the Intravenous Administration of Nicotine

The investigations cited above have shown that intravenous nicotine increases the secretion of epinephrine by the adrenal glands, but no studies have been made on the epinephrine concentration actually reaching the peripheral tissues. In the present study, peripheral arterial blood epinephrine levels have been estimated following the intravenous administration of increasing doses of nicotine sulfate to dogs. The blood epinephrine concentration following nicotinic stimulation has been correlated to the pressor response in order to establish more clearly the role of circulating epinephrine in this hypertensive episode.

Eleven dogs were anesthetized with 30 mg./kg. pentobarbital sodium given intravenously. Blood pressure from the femoral artery and respiration were recorded by conventional methods. Blood samples were obtained in polyethylene tubes from the opposite femoral artery by means of an indwelling catheter. Blood samples were assayed immediately for epinephrine using the bioassay method of Gaddum and Lembeck[6] as modified by Franko *et al.*[7] One ml. of whole blood was added to an automatically stimulated strip of rat uterus

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† Nonagram (0.001 μg.).

in a 3.0 ml. chamber. The method is sensitive to epinephrine concentrations as low as 1 μg./l., but will not detect epinephrine in the peripheral circulation under normal conditions. However it can be used to rapidly estimate blood epinephrine levels under conditions of stress, including the intravenous administration of nicotine.

Increasing doses of nicotine, ranging from 0.02 to 0.60 mg./kg., were given at 30- to 60-min. intervals to allow time for recovery between doses. The 2.0 and 4.0 mg./kg. doses were never repeated in the same dog. Control blood samples were obtained before injecting the nicotine. During the hypertensive episode following nicotine, samples were usually obtained during the rapid rise in pressure, at the time of maximum blood pressure, as the pressure fell, and after the pressure had returned to control levels (FIGURE 1). Epinephrine standards and whole blood samples were alternately tested on the uterine strip, and the inhibition by each was determined. Since the inhibition of the rat uterus is proportional to the log of the concentration of epinephrine,[6] the amount of epinephrine in the blood could then be calculated as epinephrine equivalent. No correction was made for norepinephrine. However, since norepinephrine is only about $\frac{1}{100}$ as effective as epinephrine on the rat uterus, and since not more than 10 to 20 per cent norepinephrine could be present, error due to this material would be negligible.

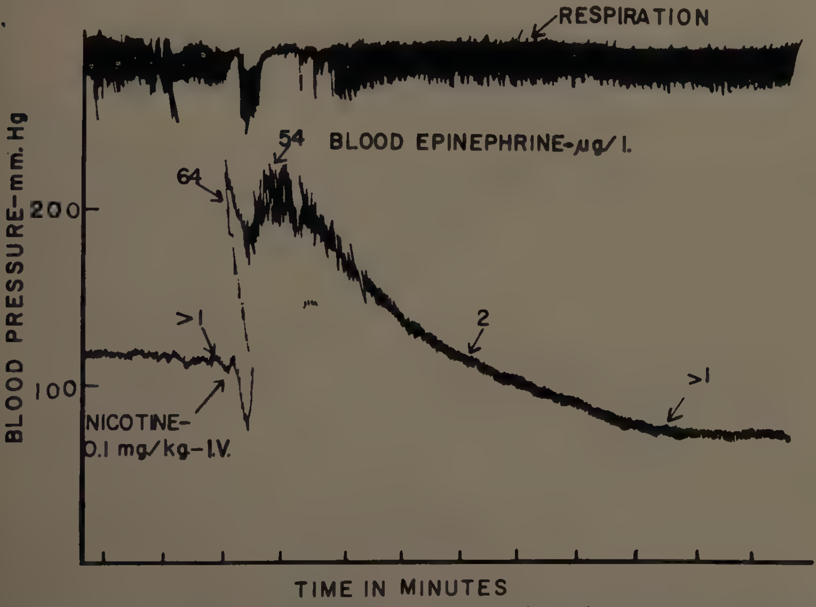

FIGURE 1. Kymograph record showing the hypertensive episode and femoral arterial blood epinephrine concentration, following the intravenous injection of 0.1 mg./kg. nicotine sulfate in a 17.2 kg. male dog. Blood epinephrine values are in μg./l. as the base in samples obtained at the points indicated by the arrows.

FIGURE 1 shows the blood epinephrine levels at several points during the hypertensive episode after the administration of 0.1 mg./kg. of nicotine. The control value was less than 1 μg./l.; the greatest concentration of 64 μg./l. was found during the ascending arm of the pressor response. The epinephrine concentration was slightly lower at the time of maximum pressure and had dropped to an undetectable concentration by the time blood pressure had returned to normal. Examination of many records indicated the maximum blood epinephrine level in femoral arterial blood occurred sometime during the ascending arm of the pressor response to nicotine. FIGURE 2 shows the blood epinephrine concentration during a similar pressor response following the intravenous administration of 0.1 mg./kg. epinephrine. There is a definite similarity in the pressor response and the blood epinephrine levels following the intravenous administration of nicotine and epinephrine.

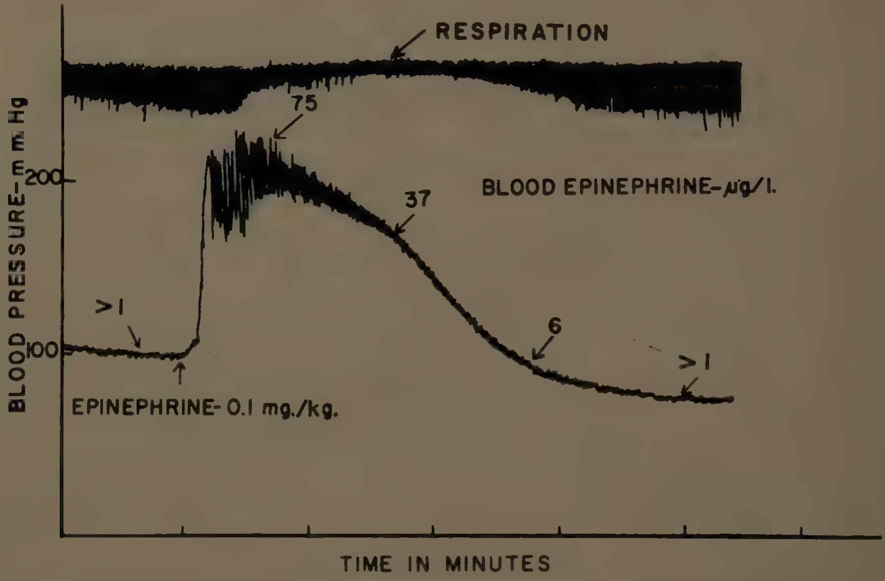

FIGURE 2. Kymograph record showing the hypertensive episode and femoral arterial blood epinephrine concentration following the intravenous injection of 0.1 mg./kg. L-epinephrine as the base in a 9.1 kg. female dog. Blood epinephrine values are in μg./l. as the base in samples obtained at the points indicated by the arrows.

TABLE 1 shows the maximum blood epinephrine levels observed after the intravenous administration of increasing doses of nicotine. The maximum levels observed are about the same over a range of 0.05 to 0.60 mg./kg. nicotine. At a dose of 2.0 mg./kg. nicotine the blood epinephrine is definitely lower than at 0.1 mg./kg. nicotine. A ganglionic-blocking dose of 4.0 mg./kg. did not elevate the blood pressure, and there was no detectable epinephrine in the peripheral circulation. The wide range in maximum blood epinephrine levels observed at each dose of nicotine is partially due to the difficulty in obtaining blood samples at precisely the time of maximum response to the intravenously administered nicotine.

TABLE 2 shows arterial blood epinephrine levels during hemorrhagic shock and after administration of drugs known to stimulate the adrenal medulla.

These data indicate that nicotine is one of the most effective compounds for releasing epinephrine from the adrenal glands. In fact, nicotine produces the highest blood epinephrine level obtained by any procedure, except by the intravenous administration of epinephrine itself.

FIGURE 3 shows there is a definite relationship between epinephrine concentration in peripheral arterial blood reaching the tissues and the pressor response following the intravenous administration of nicotine over a wide range of doses. Again it is interesting to note that a 4.0-mg./kg. dose of nicotine, which does not elevate the blood pressure, does not produce a detectable increase in blood epinephrine. Previous work has shown that the hypertensive episode following the intravenous administration of nicotine is due to the stimulation of the vasomotor center, autonomic ganglia,[8] and the adrenal medulla.[2] The present experiments definitely show that this last component, the stimulation of the adrenal medulla and the appearance of massive quantities of epinephrine in the peripheral circulation, is one of the most important factors in producing this hypertensive episode.

TABLE 1

EFFECT OF INCREASING DOSES OF NICOTINE ON BLOOD EPINEPHRINE CONCENTRATION AND BLOOD PRESSURE OF THE DOG

	Control	Dose of nicotine sulfate—mg./kg. I.V.							
		0.02	0.05	0.07	0.10	0.20	0.60	2.0	4.0
Blood epi.—μg./l.	<1	11	27	23	37	30	37	18	<1
Blood epi.—μg./l. (range)		5–36	4–40	20–26	22–112	16–46	26–68	10–34	
Number of determinations	10	9	3	2	10	2	7	3	4
B.P. increase—mm. Hg	0	41	122	112	96	120	124	114	3

TABLE 2

EPINEPHRINE LEVELS IN PERIPHERAL ARTERIAL BLOOD OF DOGS UNDER VARIOUS CONDITIONS OF STRESS

Experimental condition	Blood epinephrine μg./l. as the base	Number of determinations
Nicotine sulfate 0.1 mg./kg.	37	10
Hemorrhagic shock, bled to pressure of 40 mm. Hg	29	9
Histamine, 0.6 mg./kg. I.V., blood samples at maximum hypotensive effect	26	4
Epinephrine, single dose of 0.005 mg./kg.	37	12
Epinephrine, constant infusion of 0.005 mg./kg./min.	75	8
Acetylcholine, 0.025 mg./kg. I.V., blood samples at maximum hypotensive effect	7	4

Most early investigations, including those of Cannon[9] indicated that the epinephrine content of peripheral blood, especially during quiescence, was too low to be estimated. With the advent of improved bioassay and fluorimetric methods during the last decade, many studies on the catecholamine content of peripheral blood have been reported. The values are variable, but most studies show the epinephrine content is less than 1 μg./l., and the norepinephrine values are usually in the range of 1 to 4 μg./l.[10,11] In a carefully controlled study, Holzbauer and Vogt[12] found the epinephrine content of peripheral dog plasma to be in the range of 0.04 to 0.25 μg./l. Our studies support these conclusions. Many of the rat uteri used for bioassay would detect epinephrine in the range of 1 μg./l.; yet we seldom, if ever, detected any epinephrine in control blood samples. However, following the intravenous administration of 0.1 mg./kg. of nicotine sulfate or epinephrine, easily detectable quantities of epinephrine appeared in the peripheral arterial blood. These samples were usually bioassayed at a dilution of 1:3, but in some cases dilutions of 1:10 or 1:20 were required for assay because the lower dilutions completely inhibited the uterine strips. When epinephrine was added to shed blood in concentrations of 20 to 80 μg./l., recovery was in the range of 93 to 99 per cent.

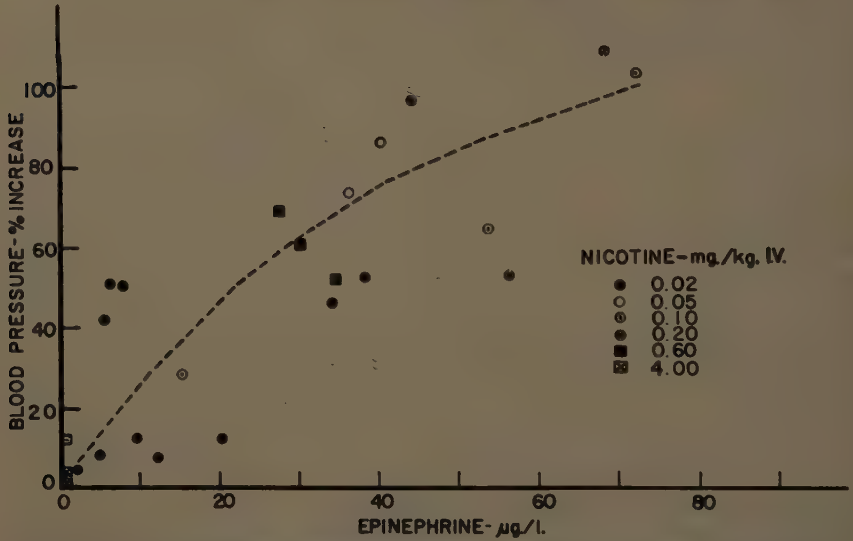

FIGURE 3. The relationship between the pressor response and maximum arterial blood epinephrine concentration, following the intravenous administration of nicotine sulfate in six dogs. Reproduced by permission from *Archives internationales de pharmacodynamie et de thérapie*.[17]

Smoking and the Urinary Excretion of Catecholamines in Man

The 3 to 4 mg. of nicotine absorbed while smoking a cigarette produces sympathomimetic effects in man, including a lowered skin temperature, increased pulse rate, elevated blood pressure, and increased metabolic rate.[13] Experiments on dogs have shown that intravenous nicotine readily mobilizes the epinephrine of the adrenal medulla, but the bioassay method used for the estimation of epinephrine was not believed to be sufficiently sensitive or practical for estimating material in peripheral blood following the milder sympathetic stimulation obtained during smoking. Euler and Zetterstrom[14] have found that epinephrine and norepinephrine are excreted to the extent of 2.3 and 3.8 per cent respectively after they were given subcutaneously in man.

Thus if the secretion of epinephrine by the adrenal medulla is a significant factor in producing the sympathomimetic effects that occur during smoking, there should be an increased excretion of these catecholamines while smoking.

The urinary excretion of epinephrine and norepinephrine was determined in a group of young, adult males during a 2-hour period in which they refrained from smoking followed by a 2-hour period of heavy smoking. The 2-hour urine samples were collected at the end of each period, acidified, the catecholamines adsorbed on aluminum oxide, eluted, and estimated by the fluorometric procedure of Euler and Floding.[15] Epinephrine and norepinephrine were oxidized to their respective adrenochromes with potassium ferricyanide, and the fluorescence read against freshly prepared standards on the Beckman DU Spectrophotometer, equipped with photomultiplier and fluorescence attachments. Tests for the significance of the data were made by Fisher's[16] method for small samples.

TABLE 3
SMOKING AND CATECHOLAMINE OUTPUT IN 11 SUBJECTS
Fluorometric Method

	Epinephrine, ng./min.			Norepinephrine, ng./min.			Cigarettes/2 hr.
	Control	Smoking	Diff.	Control	Smoking	Diff.	
Mean ± S.E.	8.2 ± 1.4	10.1 ± 1.6	+ 1.9 ± 0.43 P = 0.001	31.8 ± 3.3	29.8 ± 2.6	− 2.0 ± 1.5 P = 0.22	6.4

All values are given with ng./min. as the base (1 ng. = 0.001 μg.). Reprinted by permission from the *Journal of Applied Physiology*.[18]

TABLE 4
EFFECT OF SMOKING ON CATECHOLAMINE OUTPUT IN NINE SMOKERS
ESTIMATED BY BIOASSAY AND BY FLUOROMETRY

	Epinephrine, ng./min.						Norepinephrine, ng./min.			Cigarettes
	Bioassay			Fluorometry			Fluorometry			
	Control	Smoking	Diff.	Control	Smoking	Diff.	Control	Smoking	Diff.	
Mean ± S.E.	7.8 ± 1.5	11.4 ±2.2	+ 3.7 ±0.96 P = 0.001	10.3 ± 2.0	15.7 ±2.8	+ 5.4 ±1.4 P = 0.005	35.0 ± 2.8	36.3 ±2.3	+ 1.3 ±2.2 P = 0.6	4.6

All values are given with ng./min. as the base (1 ng. = 0.001 μg.). Reprinted by permission from the *Journal of Applied Physiology*.[18]

TABLE 3 shows the catecholamine output in 11 smokers during a 2-hour control period followed by 2 hours of heavy smoking. The increase in epinephrine output was small, but consistent and significant. The output of norepinephrine was more variable and showed a mean decrease of 2.0 ± 1.5 ng./min., which was not significant. Although the fluorometric method used for these determinations is considered the most specific of these procedures, the rather small increase in epinephrine observed during smoking made it advisable to repeat the above experiment, estimating the epinephrine by both bioassay, using the rat uterus, and by fluorometry. TABLE 4 shows the results obtained in a second group of 9 students when assays were done by the two procedures on aliquots of the urine eluates. When determined by bioassay, the mean increase in epinephrine excretion was 3.7 ± 0.9 ng./min.; by the fluorometric procedure, the increase was 5.5 ± 1.4 ng./min. The increases by both methods are statistically significant. The norepinephrine output, estimated by fluorometry only, showed a slight increase in this series, which was not significant. The epinephrine values obtained by the fluorometric method are higher than those obtained by bioassay. This is in agreement with most similar studies and probably can be attributed to the fact that some biologically inactive oxidation products of the catecholamines do fluoresce.

In these two series of experiments, in which individuals who normally smoke served as their own controls, there was a significant increase in the urinary excretion of epinephrine during heavy smoking. The increases of 1.9 and 5.4 ng./min. while smoking represent 22 and 52 per cent, respectively, over the control level. The physiological significance of this increase cannot be accurately assessed, but the magnitude is in keeping with other sympathomimetic effects known to occur while smoking. Thus the stimulation of the adrenal medulla and the release of epinephrine undoubtedly contributes to the various sympathomimetic effects observed while smoking.

References

1. Dale, H. H. & P. P. Laidlaw. 1912. The significance of the suprarenal capsule in the action of certain alkaloids. J. Physiol. **45:** 1–26.
2. Van Slyke, C. B. & P. S. Larson. 1950. Observations on the role of the adrenal medulla in the blood pressure response to nicotine. J. Pharmacol. **98:** 400–404.
3. De Schaepdryver, A. F. 1959. Physiopharmacological effects on suprarenal secretion of adrenaline and noradrenaline in dogs. Arch. intern. pharmacodynamie. **121:** 222–253.
4. Hazard, R., M. Beauvallet & S. Larno. 1957. Teneur du sang surrénal en adrénaline et en noradrénaline chez le chien soumis à l'action de la nicotine. Compt. rend. soc. biol. **151:** 210–212.
5. Malmejac, J., G. Neverre & M. Bianchi. 1957. Analyse des actions centrales et periphériques de la nicotine sur l'activitie médullosurrenale. Compt. rend. soc. biol. **151:** 707–710.
6. Gaddum, J. H. & F. Lembeck. 1949. The assay of substances from the adrenal medulla. Brit. J. Pharmacol. **4:** 401–406.
7. Franko, B. V., A. D. Bragg & D. T. Watts. 1957. A rapid bioassay for the estimation of epinephrine in peripheral blood. Arch. intern. pharmacodynamie. **111:** 123–133.
8. Langley, J. N. & W. L. Dickenson. 1889. On the local paralysis of peripheral ganglia and on the connection of different classes of nerve fibers with them. Proc. Roy. Soc. London. **B46:** 423–431.
9. Cannon, W. B. 1929. Bodily Changes in Pain, Hunger, Fear, and Rage. (Reprinted, 1953.) Branford. Boston, Mass.
10. von Euler, U. S. 1956. Noradrenaline. Thomas. Springfield, Ill.
11. Manger, W. M. 1959. Chemical Quantitation of Epinephrine and Norepinephrine in Plasma. Thomas. Springfield, Ill.
12. Holzbauer, M. & M. Vogt. 1954. The concentration of adrenaline in the peripheral blood during insulin hypoglycaemia. Brit. J. Pharmacol. **9:** 249–252.
13. Roth, G. M. 1951. Tobacco and the Cardiovascular System. Thomas. Springfield, Ill.
14. von Euler, U. S. & B. Zetterstrom. 1955. The role of amine oxidase in the inactivation of catechol amines injected in man. Acta Physiol. Scand. Suppl. 33. **118:** 26–31.
15. von Euler, U. S. & I. Floding. 1955. A fluorimetric micromethod for differential estimation of adrenaline and noradrenaline. Acta Physiol. Scand. Suppl. 33. **118:** 45–56.
16. Fisher, R. A. 1955. Statistical Methods for Research Workers. Hafner. New York, N.Y.
17. Watts, D. T. 1956. Arch. intern. pharmacodynamie. **105:** 403.
18. Watts, D. T. 1956. J. Appl. Physiol. **9:** 275.

Part III. The Influence of Nicotine and Smoking on the Peripheral Circulation

THE ACTION OF NICOTINE ON THE PERIPHERAL CIRCULATION

J. Harold Burn
Balliol College, University of Oxford, Oxford, England

Nicotine has long been known to cause a rise of blood pressure, but this action has been generally believed to be due to the stimulation of sympathetic ganglia with consequent discharge of impulses along postganglionic fibers, and also to the stimulation of the adrenal medulla, with consequent discharge of epinephrine. Nicotine has, however, a peripheral constrictor action that was described in the vessels of the frog by Handovsky and Pick (1913). This action, like the accelerator action of nicotine on the heart, is shared by acetylcholine.

In the ordinary way acetylcholine is a vasodilator substance, as Dale (1914) showed by testing its action in the vessels of the rabbit ear, but after some hours of perfusion acetylcholine causes constriction (Burn and Robinson, 1951). Kottegoda (1953) studied the constrictor actions of both nicotine and acetylcholine in rabbit ear vessels. He showed that the dilator action of acetylcholine was promptly converted to a constrictor action by atropine and that, in the presence of hexamethonium, both nicotine and acetylcholine lost their constrictor effect. Kottegoda also made two other significant observations. He showed that in the presence of tolazoline (Priscoline*), which converts the constrictor action of epinephrine in the rabbit ear vessels to a dilator action, the constrictor action of nicotine and the constrictor action of acetylcholine were also converted to dilator actions. This suggested that nicotine caused constriction by liberating epinephrine or norepinephrine from sites in or near the vessel wall. It further suggested that acetylcholine caused constriction in the same way. The other interesting observation made by Kottegoda was that the constrictor action of nicotine was observed only in the skin vessels of the rabbit ear and that, if he first removed the skin and perfused the network of vessels lying on the cartilage below, nicotine then had no constrictor action.

Action of Reserpine on Vessels

The observation that the arteries and veins contain norepinephrine was made by Schmiterlöw in 1948. My associates and I decided to determine what effect the administration of reserpine had upon the amount of norepinephrine present. For these experiments we used rabbits. First, we removed the thoracic aorta from a series of normal rabbits. We ground the tissue and made an extract, and tested the extract on the blood pressure of the rat. We found that the extract caused a rise of pressure like that caused by norepinephrine, and the mean amount present was approximately 0.5 μg./gm. When we took a series of rabbits injected with 5 mg./kg. reserpine and sacrificed them 24 hours later, extracts made from the aortas of these rabbits contained only traces of norepinephrine too small to be measured accurately. We then perfused the ears of these rabbits and discovered that neither nicotine nor acetylcholine in the presence of atropine had any constrictor action. This suggested that the injection of reserpine had not only removed norepinephrine from the rabbit aorta, but had also removed it from structures in the rabbit's ear. To dissect the vessels from the ear seemed too difficult, and therefore we decided to extract the skin of the ear. We first did this in normal rabbits, and then in rabbits treated with reserpine. In the former we found about 0.1 μg./gm. and, in the latter, we found only traces.

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* CIBA Pharmaceutical Products, Inc., Summit, N. J.

These results seemed to us to complete the evidence for the statement that the constrictor action of nicotine and of acetylcholine in the rabbit's ear was due to the release of norepinephrine (Burn and Rand, 1958).

Chromaffin Cells

At first, we concluded that the norepinephrine released by nicotine was actually present in the vessel walls. There was, however, another possibility. Nordenstam and Adams-Ray (1957) described the occurrence of chromaffin cells in human skin. Chromaffin cells are cells that stain in a manner similar to the cells of the adrenal medulla. Now it is known that both nicotine and acetylcholine release epinephrine and norepinephrine from the cells of the adrenal medulla. We wondered whether it was possible that there were chromaffin cells in the skin, and whether nicotine released norepinephrine from these cells. We therefore asked E. H. Leach, of the Department of Physiology in Oxford University, to stain some rabbit ears according to the methods described by Nordenstam and Adams-Ray. We gave Leach ears from normal rabbits and also ears from rabbits injected with reserpine, and we did not tell him which ears were which. However, he was able to distinguish the normal ears as containing chromaffin cells and to distinguish the ears from the rabbits injected with reserpine as not containing these cells, or only an occasional cell. His results, therefore, opened up the possibility that the site of the store from which nicotine released norepinephrine (or perhaps epinephrine) was the chromaffin cells.

The Pilomotor Action of Nicotine

In order to obtain further evidence as to chromaffin cells, I refer to other observations made on the cat's tail. It is well known that, when the sympathetic chain is stimulated, the hairs on the tail stand up. The erection of the hairs is caused by the arrectores pili, the muscles in the skin of the tail. Sympathetic impulses going to the muscles make the muscles contract. The process of erection of the hair can be studied by removing most of the hair from the tail with clippers, so that only a few tufts remain. Now it was observed by Coon and Rothman (1940) that nicotine, when injected into the skin at the base of a tuft, would cause erection of the tuft. Thus we now have three actions of nicotine that are peripheral actions and therefore cannot be ganglionic actions, but nevertheless resemble the effect of sympathetic stimulation. The first is acceleration of the heart, the second is constriction of the vessels of the rabbit ear, and the third is erection of the hair of the cat's tail. We have seen that both the heart and the skin of the rabbit ear contain norepinephrine, which can be extracted. We therefore extracted the skin of the cat's tail and found that it contained 0.35 μg./gm. norepinephrine. We then took cats treated with reserpine, and found that in them the amount of norepinephrine that could be extracted was greatly reduced (0.057 μg./gm.); further, in such cats the action of nicotine in causing erection of the hair was very small.

We have also seen that chromaffin cells have been found in the heart and in the skin of the rabbit ear. In the skin of the cat's tail they are much more plentiful than in the skin of the rabbit's ear, and Leach informed us that in the skin of the cat's tail the chromaffin cells could be seen in the very middle of the arrectores pili muscles themselves. These chromaffin cells disappeared when the cats were treated with reserpine. A fourth example of the same phenomenon is to be found in the nictitating membrane, which can be isolated together with its nerve and studied outside the body (Thompson, 1958). Here again is an organ with a sympathetic nerve supply that is free of ganglia and can be stimulated by nicotine. It contains chromaffin cells. If prepared from a cat that has been treated with reserpine, the membrane no longer can be stimulated to contract by nicotine, and it no longer contains chromaffin cells (Burn *et al.*, 1959).

The accumulation of evidence from these four organs respecting the peripheral action of nicotine strongly suggests that nicotine produces effects similar to sympathetic effects because it causes a discharge of norepinephrine and, perhaps, of epinephrine from chromaffin cells. However, it is impossible to say that this point is proved.

Cholinergic Fibers

Our results suggest that there may be a widespread system of chromaffin cells in close relation to the sympathetic nerves, or at least in close relation to some of them. We have studied the effect of stimulating sympathetic nerves in animals treated with reserpine, and we have found in all cases evidence that sympathetic nerves release acetylcholine. If this acetylcholine (which must be released in the normal animal as well as in the reserpine-treated animal) then acts on chromaffin cells and liberates norepinephrine, we have a second form of adrenergic mechanism to take its place side by side with the classic adrenergic neuron. In this new form of adrenergic mechanism a cholinergic fiber may innervate a cell which is a chromaffin cell, in a manner similar to that in which the splanchnic fibers innervate the adrenal medulla (Burn and Rand, 1959).

Action of Nicotine on the Blood Vessels

Whatever truth there is in these suggestions, we have clear evidence that in vessels such as those of the rabbit ear, the action of nicotine depends on the discharge of norepinephrine from a store near to or in the vessel walls. It seems to be this action that is responsible for the sharp fall of skin temperature caused by smoking cigarettes. Probably it is this action of nicotine that explains the exacerbation of conditions such as thrombo-angiitis obliterans. It is an action suggesting that peripheral disease, such as Raynaud's disease and

Buerger's disease, would benefit from the continued use of daily doses of reserpine, although these should certainly be very small because of the risk of causing mental depression.

Action of Nicotine in the Brain

I conclude this paper by pointing out what seems to be a corollary of the matters I have discussed. We have seen that nicotine liberates norepinephrine from stores in the heart and in the vessel walls. Vogt (1954) has mapped out the distribution of norepinephrine in the brain, finding large amounts in the hypothalamus. Holzbauer and Vogt (1956) have shown that when animals are treated with reserpine the norepinephrine diminishes greatly in quantity, and we know that the animals then become depressed. Hence there is some reason to think that the norepinephrine plays a part in the brain, contributing somehow to maintaining a normal lively mood. This is particularly emphasized by the observation that the administration of the amino acid dopa (dihydroxyphenylalanine), which is a precursor of norepinephrine, arouses mice from their depression when they have been treated with reserpine (Carlsson *et al.* 1957).

It seems to me extremely likely that the pleasure of smoking is derived in part from the release of norepinephrine from its store in the brain by nicotine, the release giving an increased feeling of cheerfulness and a sense of relief from fatigue.

References

Burn, J. H., E. H. Leach, M. J. Rand & J. W. Thompson. 1959. Peripheral effects of nicotine and acetylcholine resembling those of sympathetic stimulation. J. Physiol. **148:** 332.

Burn, J. H. & M. J. Rand. 1958. Noradrenaline in artery walls and its dispersal by reserpine. Brit. Med. J. **i:** 903.

Burn, J. H. & M. J. Rand. 1959. Sympathetic postganglionic mechanism. Nature. **184:** 163.

Burn, J. H. & J. Robinson. 1951. Reversal of the vascular response to acetylcholine and adrenaline. Brit. J. Pharmacol. **6:** 110.

Carlsson, A., M. Lindgvist & T. Magnusson. 1957. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature. **180:** 1200.

Coon, J. B. & S. Rothman. 1940. Nature of pilomotor response to acetylcholine; some observations on pharmacodynamics of skin. J. Pharmacol. **68:** 301.

Dale, H. H. 1914. The action of certain esters and ethers of choline and their relation to muscarine. J. Pharmacol. **6:** 147.

Handovsky, H. & E. P. Pick. 1913. Untersuchungen über die pharmakologische Beeinflussbarkeit des pieripheren Gefässtonus des Frosches. Arch. Exptl. Pathol. Pharmakol. **71:** 89.

Holzbauer, M. & M. Vogt. 1956. Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. J. Neurochem. **1:** 8–11.

Kottegoda, S. R. 1953. The action of nicotine and acetylcholine on the vessels of the rabbit ear. Brit. J. Pharmacol. **8:** 156.

Nordenstam, H. & J. Adams-Ray. 1957. Chromaffin granules and their cellular location in human skin. Z. Zellforsch. **45:** 435.

Schmiterlöw, C. G. 1948. The nature and occurrence of pressor and depressor substances in extracts from blood vessels. Acta Physiol. Scand. **16:** Suppl. 56.

Thompson, J. W. 1958. Studies on the responses of the isolated nictitating membrane of the cat. J. Physiol. **141:** 46–72.

Vogt, M. 1954. The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. J. Physiol. **123:** 451.

THE ACUTE EFFECT OF CIGARETTE SMOKING ON THE DIGITAL CIRCULATION IN HEALTH AND DISEASE*

Jack Freund and Claire Ward

Department of Pharmacology and Medicine, Medical College of Virginia, Richmond, Va.

The acute effects of cigarette smoking on the peripheral circulation have been of interest since the early studies of Bruce *et al.*[1] in 1909. In the intervening years methods used to measure the effect have included plethysmography, skin temperature and calorimetry, direct observation of capillary circulation, thermal conductance, and the use of radioactive isotopes. Although these procedures are of necessity indirect and all have merits and limitations,[2] the data they furnish are indicative of blood flow by reflecting a change in the quality and quantity of tissue being measured.

In the studies reported here, it was expected that reliable data could be obtained by properly framing the question of nature[3] as follows: What are the acute changes occurring in the digits following cigarette smoking in health and in advanced peripheral vascular disease?

Several methods were employed to answer this question. These included plethysmography, skin temperature determinations, and skin clearance of radiosodium. It was anticipated that the findings would be complementary and, thus, would broaden the scope of the study. Determinations of arterial and venous oxygen saturation and lactic acid were added to these techniques to ascertain the role, if any, of shunts during smoking. To insure further a valid inference and reduce the effect of individual variation in digital response, all results were subjected to statistical analyses.

MATERIALS AND METHODS

Subjects

All subjects were habitual smokers. Thirty-three studies were performed on 15 healthy male penitentiary inmates all below 35 years of age, and 26 studies on 14 patients with peripheral vascular disease. The average age of the latter patients was 65. Arteriosclerosis obliterans was present in 12 patients and Raynaud's phenomenon and Buerger's disease in each of the remaining two. These were all individuals with advanced disease, as evidenced by absent foot pulses and atrophic digital skin changes.

Methods

Skin temperature. All studies were performed in a room where the temperature was maintained at 25° C. ±1° and the humidity at 40 per cent. Skin temperature was recorded on a Brown potentiometer throughout the experiment. This instrument is a constantly recording instrument with a high-gain amplifier resulting in an over-all sensitivity of ±0.25° C. Skin temperatures were measured by a Constantan-copper thermocouple imbedded in a plastic rod attached to a spring device[4] that maintained a constant pressure at the skin contact.

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Plethysmography. The electronic pneumoplethysmograph described by Winsor[5] was used to determine volume changes in fingers and toes. Its maximum sensitivity is a 50-mm. movement of the stylus for a 5-cu. mm. volume change in the pneumatic system. For recording, this instrument was connected to the DC amplifier of a Sanborn Direct Writing Electrocardiograph. The Sanborn was modified to record at 2.5 mm. and 25.0 mm./sec. To obtain prompt venous occlusion for the cup around the finger (or ankle, for the toe), pressure gauges in series were attached to a compressed air tank enabling a 60-mm. Hg pressure to be instantaneously delivered to the occluding cuff. Plastic finger cups were attached to the plethysmograph by a 7-foot plastic tube with a 1-mm. lumen. The volume of each cup was 14 cc.

Radioactive sodium-24 for clearance from the skin. The instruments used to determine skin clearance were the nuclear D-S scintillation counter and count-rate meter, and the Esterline-Angus recorder.

Biochemical determinations. Lactic acid.[6,7] Oxygen saturations were determined by the Beckman spectrophotometric method.

Protocol

The subjects were brought to the constant temperature room at the Medical College of Virginia at 8:30 A.M. All were instructed not to smoke for at least 12 hours prior to the experiment and were in the fasting state. The procedures were carefully explained to allay any apprehension. The subjects then dressed in pajamas with short sleeve tops and bottoms, arranged so that there was easy access to the femoral artery. Four cc. of finger and toe tissue were measured by the displacement of merthiolate in a calibrated vial. An area over the femoral artery opposite that of the toe being studied was shaven.

Under aseptic technique and after skin infiltration with Procaine, a Cournand needle was placed in the femoral artery opposite the toe studied. Plethysmographic cups were placed on a toe and finger and were sealed with calking compound. An indwelling Reilly needle was placed in a dorsal vein of the hand or foot draining the digits to be studied.

A thermocouple placed in contact with the dorsal aspect of the digits recorded skin temperatures throughout the remainder of the experiment.

After the calking compound was rechecked, control plethysmographic records were made of a finger and toe in normal subjects, and of toes alone in patients with disease, at speeds of 2.5 mm. and 25.0 mm./sec. (five determinations at each speed were performed).

Control studies were performed to measure the clearance of 0.05 cc. of sodium-24 (or sodium-22) injected intracutaneously at the dorsal aspect of the distal phalanx of an adjacent finger or toe. Finally, a 10-cc. blood sample was taken from the femoral artery or foot veins as a control observation.

Studies of sham smoking were performed only in the normal subjects after control procedures. During sham smoking, the subject was advised to puff on an unlit filter-tip cigarette for 10 min. as if he were actually smoking. A filter-tip cigarette was used to avoid the subject's contact with nicotine prior to actual smoking and yet produce the hyperventilation effects associated with smoking.

After all controls and sham procedures, the subjects were allowed to return to a basal state (15 to 30 min.). Each subject then smoked a filtered cigarette at his normal rate of smoking and was allowed two cigarettes in succession if he so desired. A protocol similar to the control procedures was then followed. Lactic acid determinations were performed as the bloods were drawn; or, if necessary, the protein-free filtrates were held over to the following day. Oxygen saturations were determined on the same day the blood was drawn.

Technical Determinations

Skin temperature. Since records were taken continually except for necessary readjustment of the thermocouple, the lowest reading per minute was used in control (presmoking), postsham, and postsmoking calculations. Because of the known variation in basal skin temperatures, the readings at 5 min. presham and presmoking were taken as control values.

Plethysmography. For finger plethysmography the occluding cuff was placed no farther than 2 cm. from the cup, and for toe plethysmography the venous occlusion cuff (an adapted blood pressure cuff) was applied at the ankle. Since it was noted that errors might result from venous congestion after more than five rapid occlusions of the finger or toe, only five readings at rapid speed were taken.

Radiosodium clearance. Sodium-24 was used on test subjects first; later, determinations were made with sodium-22 on patients with peripheral vascular disease. The half life was utilized as an index of clearance (where the counts were reduced to one half the initial count). The radioactive sodium was injected intradermally and any excess material was lightly swabbed from the skin.[8] Results were accepted only where the counts were more than twice that of the background activity.

Biochemical determinations. All were done in duplicate.

The Analysis of Variance was performed to estimate the proportion of variation within and among individuals and the temporal variation in individuals.

RESULTS

Skin Temperature

Normal subjects. It is readily apparent from FIGURE 1 that a significant decrease in postsmoking skin temperature was observed from 0 to 10 and 5 to 10 min. This indicates a definite change, with predominant values occurring in 5 to 10 min. There was no significant change in skin temperatures after sham smoking.

Patients with peripheral vascular disease. The results in these patients are plotted in FIGURE 2. When the raw data were treated statistically, no significant change was observed.

Plethysmography

Normal subjects. In 15 individuals (FIGURE 3), there was a significant decrease in finger plethysmography values following sham smoking, and in toe plethysmography following smoking. These results could not readily be explained and were, therefore, subjected to closer scrutiny. As seen in TABLE 1, normal subject No. 13 on one day had a significant decrease in digital volume

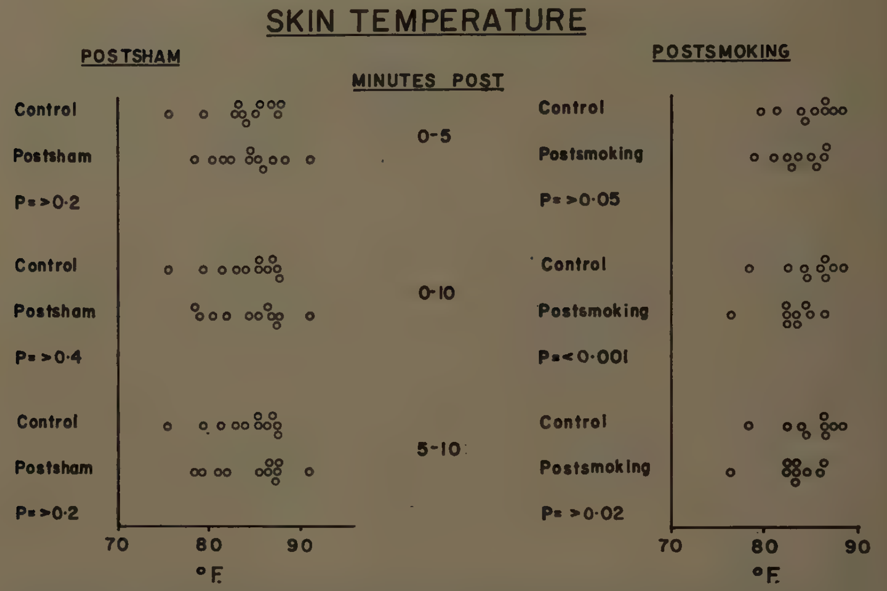


FIGURE 1. Skin temperature in normal subjects after sham and actual smoking. The most significant decrease in skin temperature occurred following actual smoking from 0 to 10 and from 5 to 10 min. No significant change was noted after sham smoking.

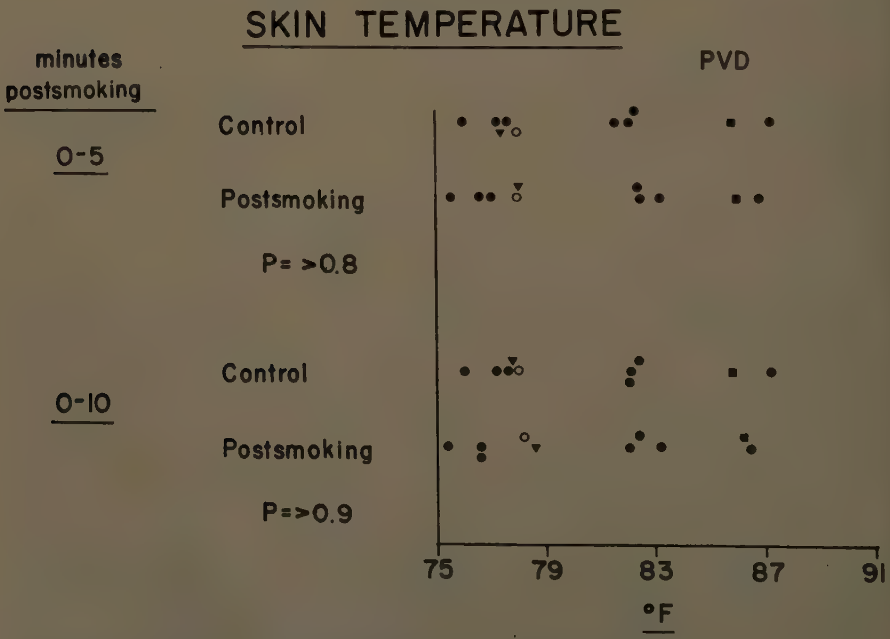


FIGURE 2. Five and 10 min. after smoking, there was no significant decrease in digital temperature in patients with marked peripheral vascular disease.

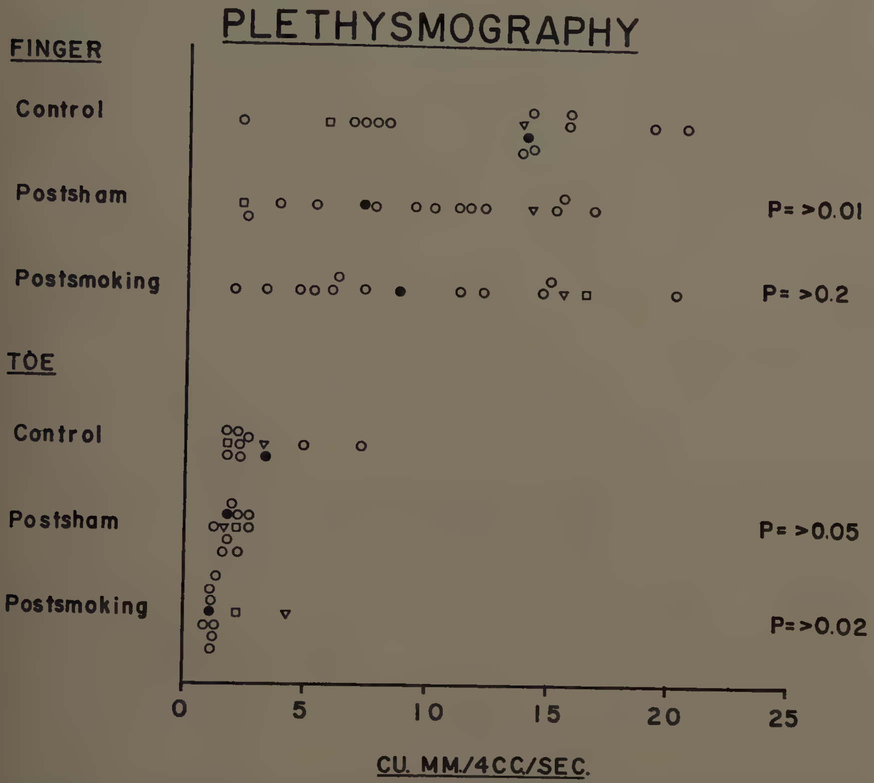


FIGURE 3. In 15 normal subjects there was a significant decrease in volume of the finger after sham smoking and in the toe after actual smoking. These values were for the group as a whole.

TABLE 1
VARIATION IN INDIVIDUALS IN FINGER PLETHYSMOGRAPHY
Significance at 5 Per Cent Level

Individual	Trial	Postsham	Postsmoking
5	b	>0.05	*<0.001*
	c	>0.9	*>0.02*
6	c	*>0.001*	*>0.02*
	d	>0.2	*>0.02*
7	b	*<0.001*	*<0.001*
	d	>0.1	>0.1
13	a	*>0.01*	>0.1
	b	>0.05	*<0.001*

TABLE 2

VARIATION IN INDIVIDUALS IN TOE PLETHYSMOGRAPHY

Significance at 5 Per Cent Level

Individual	Trial	Postsham	Postsmoking
3	a	*>0.01*	*<0.001*
	b	>0.1	*>0.01*
4	a	*>0.02*	>0.2
	b	>0.4	*>0.02*
5	b	>0.3	>0.7
	c	>0.8	>0.05
8	c	*>0.001*	*<0.001*
	d	*>0.001*	*>0.01*

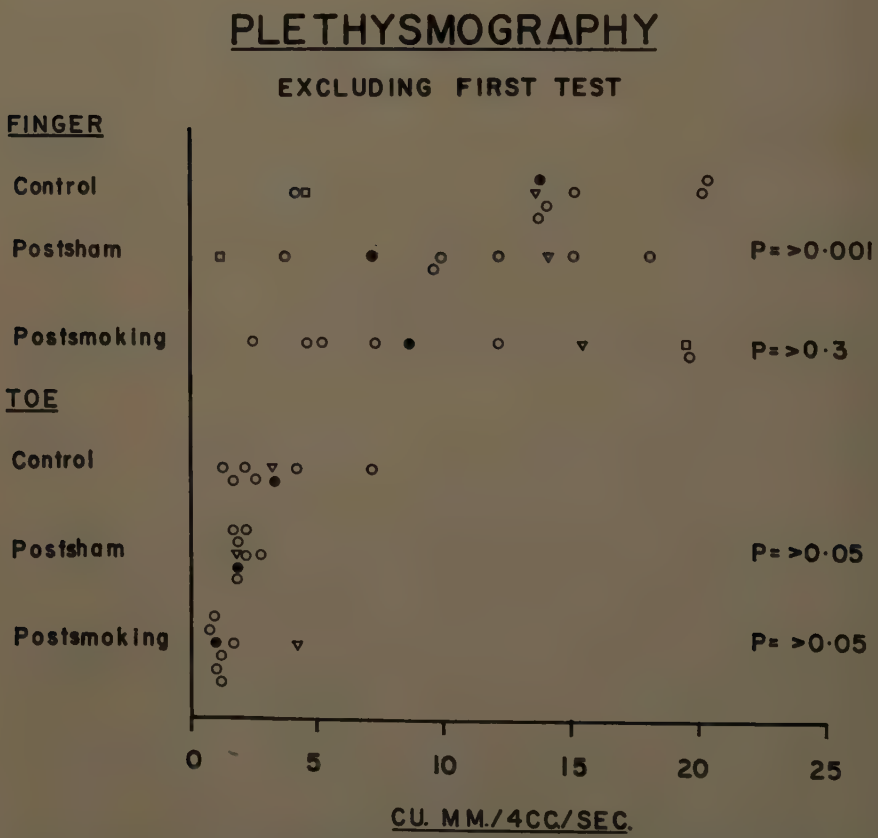


FIGURE 4. When the values for the first test are excluded from the normal group as a whole, the postsham value for finger volume remained significant and the values for toe volume were not significant. This indicates that apprehension probably caused an alteration in the post-smoking toe results.

following sham smoking and, during the next experiment, a significant decrease only after actual smoking. Similar variation occurred in toe plethysmography (TABLE 2) in other subjects. Because of the possible role of apprehension, the values for the first test were excluded. It was then noted that a significant reduction in values was observed in the finger only following sham smoking (FIGURE 4). These results led us to conclude that the day-to-day variation in normals was too marked to permit a valid comparison.

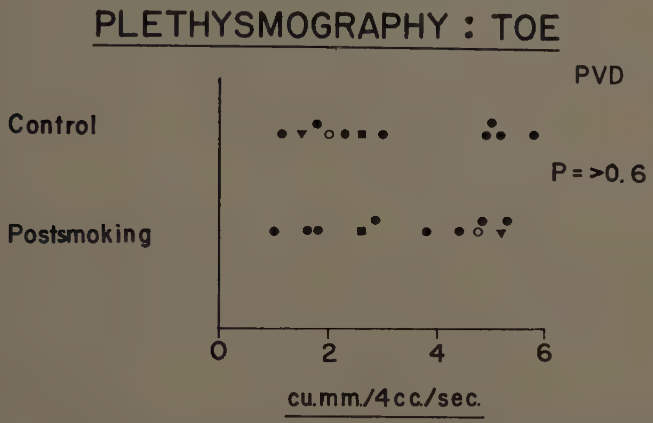


FIGURE 5. There was no significant change in toe volume in patients with marked peripheral vascular disease.

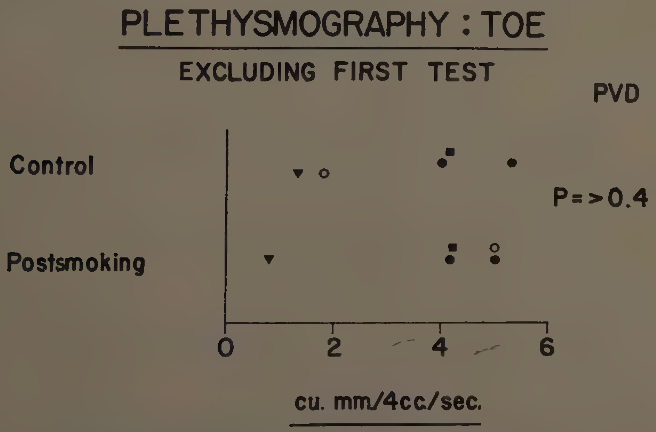


FIGURE 6. In patients with marked peripheral vascular disease, exclusion of the first test did not alter the results of toe plethysmography.

Patients with peripheral vascular disease. Since no statistically significant results were obtained following sham smoking in normals, this procedure was omitted. Studies in 11 patients were performed only in the involved digits: the toes. No significant change occurred following smoking (FIGURE 5) even when the first test was excluded (FIGURE 6).

Thus in normals using plethysmography, the variation was so great that one could not predict from day to day the results following smoking. In individuals with advanced peripheral vascular disease, there was no significant change for the group following smoking.

Radioactive Sodium Skin Clearance

Normal subjects. The results of radiosodium clearance from the skin of the digit are plotted in FIGURE 7. There was no significant increase in half-life

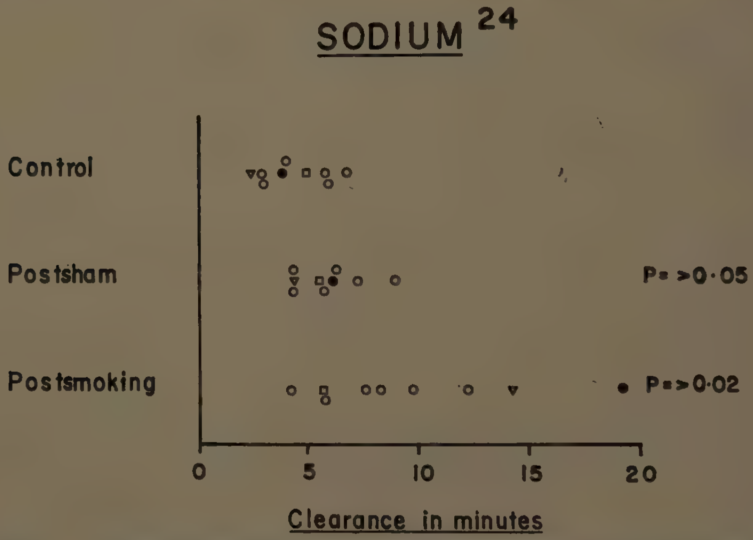


FIGURE 7. In normal subjects, there was no significant decrease in Na^{24} half life after sham smoking. A significant decrease occurred after smoking.

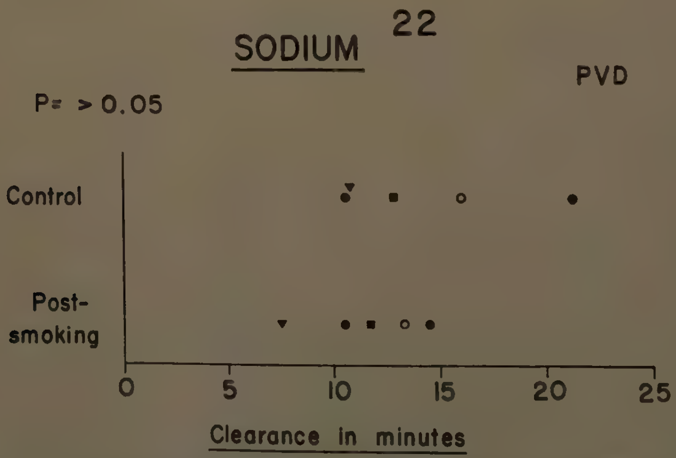


FIGURE 8. In patients with peripheral vascular disease, there was no change in the Na^{22} clearance half-life values for all patients after smoking.

values (prolongation of clearance) after smoking, as compared to control determination. No significant changes were noted after sham smoking.

Patients with peripheral vascular disease. The results in five patients can be seen in FIGURE 8. There was no change in the average clearance half-life values for *all* patients after smoking. In two individual patients with repeat tests, one test demonstrated a decrease after smoking and eight of ten tests showed an increase. For the group as a whole, however, the decrease was not significant. The probability of this difference was between 0.05 and 0.10.

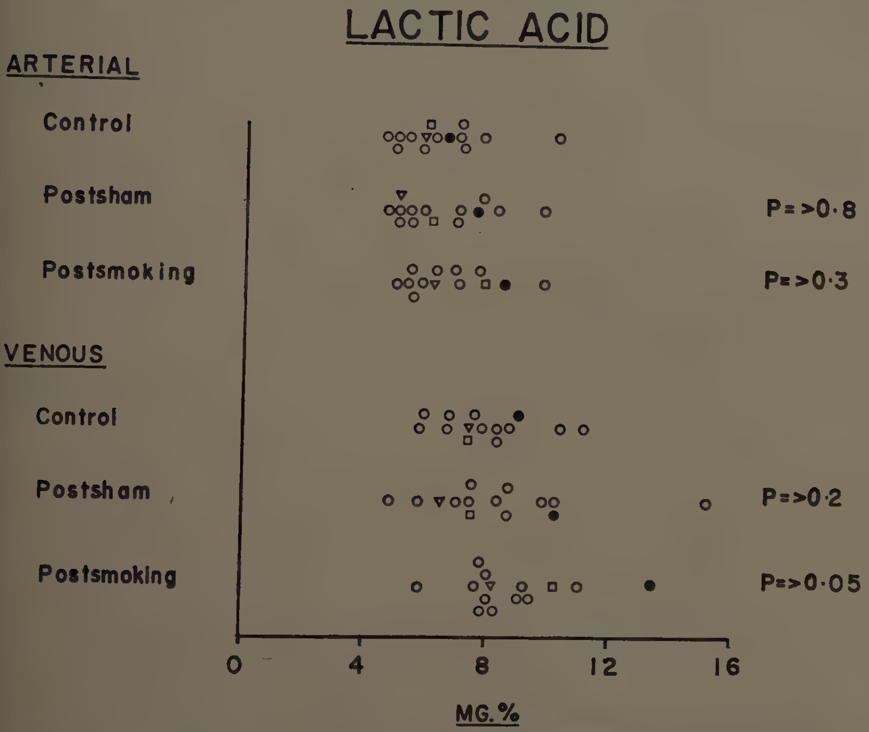


FIGURE 9. In normal subjects, there was no significant change in arterial or venous lactic acid values after smoking.

It should be mentioned that skin clearance was performed on the fingers of normal subjects and on the toes of individuals with peripheral vascular disease. However, there was a prolonged radiosodium skin clearance after smoking in normal subjects, while there was no significant change in the toes of subjects with peripheral vascular disease.

Biochemical Determinations

Lactic acid. As may be seen in normals (FIGURE 9) and in patients with peripheral vascular disease (FIGURE 10), there was no statistically significant change in either arterial or venous lactic acid values following smoking. This, of course, does not indicate that individuals did not have any decrease in lactic acid values. Four of the five patients with peripheral vascular disease consistently had a decrease in lactic acid and venous blood on repeat tests after smoking. The fifth patient had an increase on both tests. For the group as a whole, however, the decrease was not significant and the probability was between 0.05 and 0.10. Furthermore, the changes in the postsmoking concentration of lactic acid in arterial blood of patients with peripheral vascular disease followed no definite pattern. For instance, there was an increase in two patients, no change in one, and a decrease in two patients. Further, the lactic acid value in the group with disease increased in both tests of one patient, decreased in both tests of one patient, increased in one, and decreased in the other two patients.

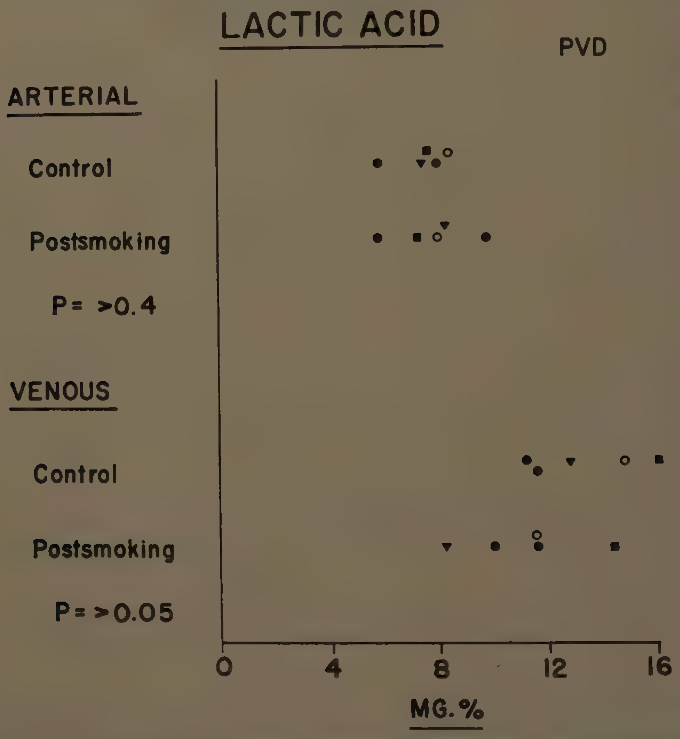


FIGURE 10. In patients with peripheral vascular disease, there was no significant change in arterial or venous lactic acid values after smoking.

Oxygen saturation. As with the lactic acid determinations, after smoking, there was no obvious pattern of change in the oxygen saturation of arterial blood. This lack of significance occurred in both normal subjects and patients with peripheral vascular disease, as seen in FIGURES 11 and 12. A significant decrease in venous oxygen saturation was seen only in normals.

There were also individual changes in the oxygen saturation of arterial blood of patients with disease. For instance, of nine tests, four showed increases and five decreases. Two patients decreased in both tests, one patient increased in both tests, and one patient both increased and decreased.

A composite analytical summary of the results of all tests is presented in TABLE 3. In normal males, a significant reduction in digital skin temperature, radiosodium skin clearance, and venous oxygen saturation was observed after cigarette smoking. The results in the entire normal group with plethysmography did show significant differences. However, the individual variability was so great that adequate prediction could not be made of future results using the same technique and the same individual. Although there was no significant change from control values in any of the parameters of measurement in the group of patients with peripheral vascular disease, this should not exclude the observations that there were changes in individuals when the results after smoking were compared to control values. It should be re-emphasized that the analysis was applied to the group as a whole.

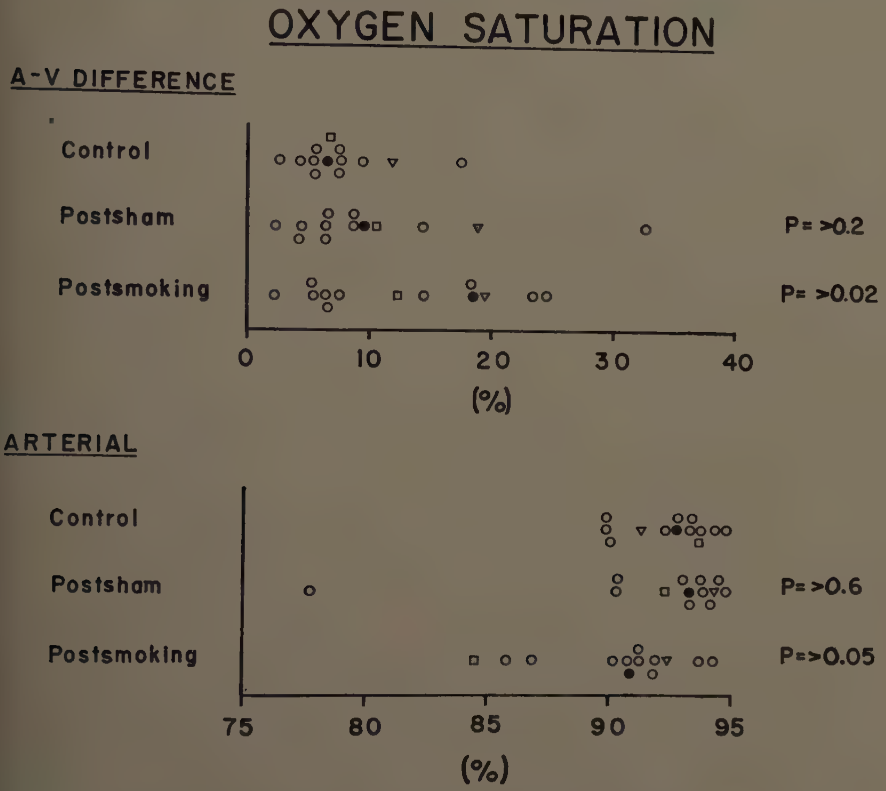


FIGURE 11. In normal subjects, there was no significant change in arterial oxygen saturation and a significant change in venous oxygen saturation after smoking. (This may be indicative of a shunt mechanism.)

DISCUSSION

Since approximately one half the volume of the fingers is made up of skin and subcutaneous tissue,[9] changes in the various test values are a reflection of alterations in the caliber of cutaneous blood vessels. Our data indicate, at least in normals, that cigarette smoking produces constriction of the arteries in the skin of the digit. Even though we made a definite attempt to allay apprehension, there was an element of digital response to the psychic stimulus of the procedure. This effect on plethysmography has been previously reported.[10,11] Thus, as noted in FIGURES 3 and 4, when one excludes the first test on plethysmography, there is a greater significance (0.001 as compared to 0.01) after sham smoking and a change in significance after actual smoking from 0.02 to 0.05. The drop in cutaneous temperature that we observed was similar to the results obtained by Roth *et al.*[12]

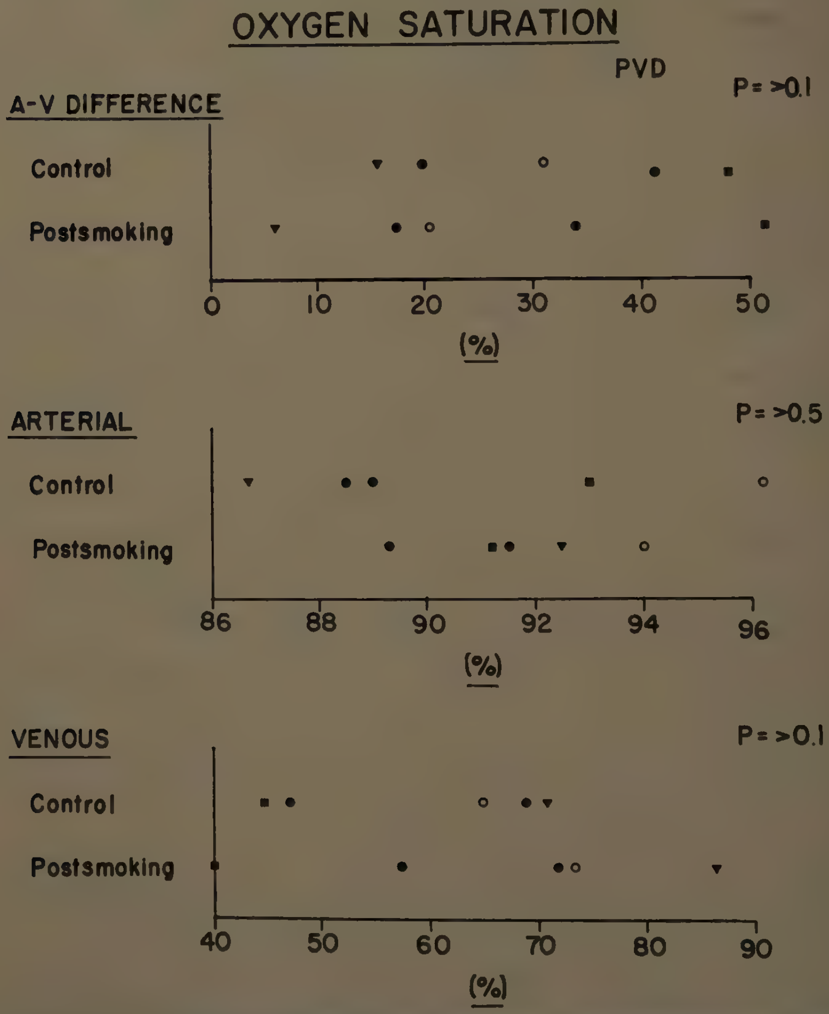


FIGURE 12. In patients with peripheral vascular disease, there was no significant change in venous or arterial oxygen saturation after smoking.

It has been postulated by Goetz[13] that the peripheral constriction caused by smoking results from two different mechanisms. The first is an immediate reflex action due to inhalation, and the second results from the pharmacological effect of nicotine. The results, with the exception of plethysmography, did not reveal any consistent change in the normals after puffing on an unlit cigarette (hyperventilation). This is essentially in agreement with the findings of Weatherby.[14] Although significant changes did occur in the group as a whole after sham smoking, as determined by plethysmography, they were extremely variable and the inconsistancies occurred on different experiment days. Our findings, therefore, are at variance with those of Mulinos and Shulman.[15] From our results, we cannot agree that "deep breathing alone can account for the greater part of the decreased blood inflow rate, the loss of hand volume, and the drop in skin temperature of the hand resulting from the inhalation of cigarette smoke."

TABLE 3
COMPOSITE CHART: POSTSMOKING
Significance at 5 Per Cent Level

Test	Normals	PVD
Na^{24}	*>0.02*	
Na^{22}		>0.05
Lactic acid		
arterial	>0.3	>0.4
venous	>0.05	>0.05
Oxygen saturation		
arterial	>0.05	>0.5
AV difference	*>0.02*	>0.1
Skin temperature		
0–5 min. post	>0.05	>0.8
0–10 min. post	*<0.001*	>0.9
Plethysmography—toe		
all tests	>0.02	>0.6
excluding first test	>0.05	>0.4

It is generally agreed that the widespread complex pharmacological effects following smoking are due to nicotine. Recent studies by Burn *et al.*[16] have indicated that the peripheral effects of nicotine are produced by the liberation of noradrenalin from a peripheral store. It is no wonder, then, that changes in digital circulation are actually so complex; they are an end result of many central and peripheral factors. Our results in normal habitual male smokers in general agree with those of Weatherby,[14] Moyer and Maddock,[17] and Stewart *et al.*[18] A decrease in venous oxygen saturation in normals was presumably a result of the vasoconstriction.

Our results of radioactive sodium clearance in normal subjects reflected a decrease in local blood flow after smoking. Friedell,[19] using radioactive iodinated albumen as an indicator of volumetric change in the extremity, found a sex difference in sensitivity to tobacco smoke. Since there was only one woman in our series, sex differences could not be compared. It is of interest that in the subjects over 35 years of age Friedell did not find any significant vasoalterative effects after smoking. These findings are similar to our results where there was decreased tissue reactivity in older patients.

The effect of the rate of smoking, as described by Shepherd,[20] and of sham smoking in normals did not appear to be a significant factor in our studies. In retrospect, this might have been more carefully controlled. However, the rate of smoking was timed in a number of individuals who were unaware of our observation. As an example, one normal subject puffed four times a minute during both sham smoking and actual smoking; another subject puffed five times a minute during sham smoking, and only four times a minute during actual smoking.

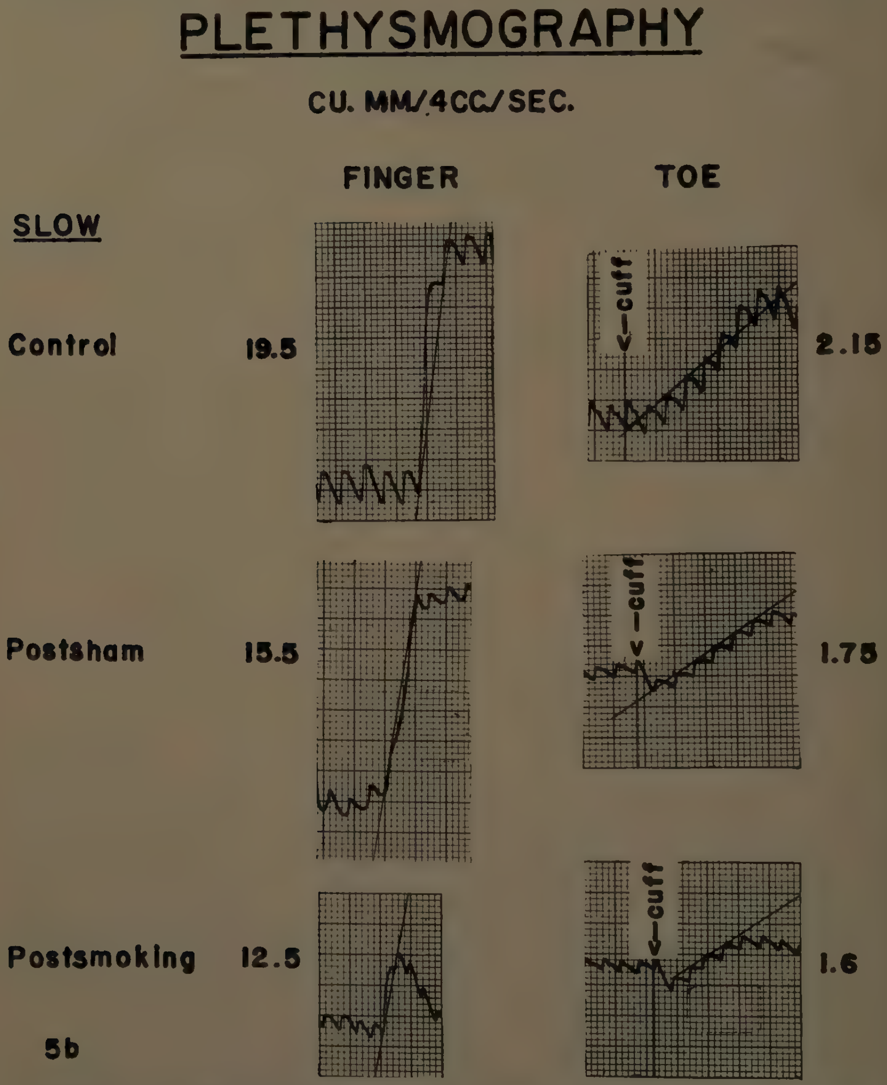
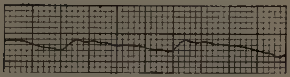
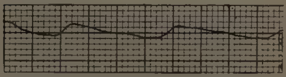
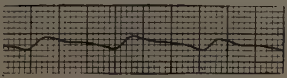


FIGURE 13. Individual plethysmographic tracings in normal subjects, demonstrating typical responses to smoking.

There was an absence of foot pulses and atrophic skin changes in the digits of all 12 patients with arteriosclerosis obliterans. Admittedly, the degree of ischemia in these individuals varied, but we feel that the group was as homogeneous as could be obtained under the circumstances.

Stewart *et al.*[18] demonstrated that the effects of smoking are quantitatively less in older age groups. The lack of response in our patients indicates a lessening reactivity of tissue in older patients with advanced superimposed disease. The failure of shunts in the ischemic limb[21] to operate in our patients may be attributed to nicotine-induced sympathetic stimulation. This then suggests that a peripheral sympathomimetic action was present. However, the degree of this effect was not sufficiently prominent to produce vasoconstriction that was statistically significant for the group as a whole.

PULSE-WAVE ANALYSIS

FINGER		Rate beats/min.	Height mm.	Area sq. in.
Control		74	4	7
Postsham		75	2.5	4
Postsmoking		96	2.5	3

6d

FIGURE 14. Tracings of digital pulse waves in normal subjects taken during plethysmography at rapid speeds. These show an increase in pulse rates after smoking and a decrease in height and area of each wave after sham and actual smoking.

Although results with plethysmography were variable in normals and in patients with disease, typical responses in some individuals were observed, as shown in FIGURE 13. The increase in pulse rate observed on pulse-wave analysis of finger plethysmography (FIGURE 14) is in general agreement with most observers.

In our studies, the skin temperature 5 min. prior to smoking and sham smoking was compared to postsmoking and postsham values, each patient and subject acting as his own control. In general, there is agreement[22] that the drop in skin temperature was of relatively short duration.

Our results should not be interpreted to mean that smoking is permissible in patients with peripheral vascular disease. A minimal reduction in blood flow in patients with ischemic limbs may produce a further reduction in tissue nutrition, and thus may be another case of the proverbial straw on the camel's back. We assume that in patients with moderate involvement the digital responses would approach those observed in our normal subjects.

In general, we have found changes in our parameters of measurement indicating digital vasoconstriction of short duration in normal subjects. In our experience, hyperventilation during smoking did not appreciably affect the test results. It should be noted that these results were obtained *after* sham smoking and not *during* the period of hyperventilation. The variability of findings with plethysmography from day to day indicated the extreme sensitivity of this type of measurement and multiple factors involved in this procedure. The findings in patients with advanced disease in the old-age group suggests a decreased reactivity of tissue. However, a sufficient degree of sympathetic stimulation was apparently present to prevent the shuntlike mechanisms from functioning.

SUMMARY AND CONCLUSIONS

(1) Multitechnical procedures were performed on the digits of 15 healthy male penitentiary inmates and 14 patients with peripheral vascular disease. These included skin temperature, digital plethysmography, radiosodium skin clearance, and AV difference of oxygen saturation and lactic acid.

(2) Thirty-three studies were performed on healthy males and 26 on patients with peripheral vascular disease.

(3) In normal males, a significant reduction in digital skin temperature, radiosodium clearance, and venous oxygen saturation was observed after cigarette smoking. There was no statistical change after sham smoking. During plethysmography in the normals, there was a marked individual variation, although significance was obtained in the group as a whole.

(4) Smoking produced no significant change from control values in the group of patients with marked peripheral vascular disease. This was explained on the basis of decreased skin reactivity in these patients.

ACKNOWLEDGMENTS

We are indebted to Bertram L. Hanna of the Department of Biology and Genetics of the Medical College of Virginia for statistical treatment of the data.

REFERENCES

1. BRUCE, J. W., J. R. MILLER & R. HOOKER. 1909. The effect of smoking upon the blood pressures and upon the volume of the hand. Am. J. Physiol. **24:** 104–116.
2. BURTON, A. C. 1954. Critical survey of methods available for the measurement of human peripheral blood flow. *In* Peripheral Circulation in Man. Ciba Symposium. : 3–19.
3. SNYDER, L. H. 1951. Framing sensible questions of nature. Proc. Okla. Acad. Sci. **32.**
4. EVANS, E. I., J. W. BROOKS, F. H. SCHMIDT, R. C. WILLIAMS & T. HAM, JR. 1955. Flash burn studies on human volunteers. Surgery. **37:** 280–297.
5. WINSOR, T. 1953. A sensitive direct writing plethysmograph. Electrical Engineering. July, 1953.
6. HAWK, P. B., B. L. OSER & W. H. SUMMERSON. 1941. Practical Phys. Chem. **569.**
7. BARKER, S. B. & W. H. SUMMERSON. 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. **138:** 535.
8. FREUND, J., L. H. WISHAM & R. S. YALOW. 1953. The effect of priscoline on the clearance of radiosodium from muscle and skin of man in normal and diseased limbs. Circulation. **8:** 89–97.
9. GRANT, R. T. & R. S. B. PEARSON. 1938. The blood circulation in the human limb; observations on the differences between the proximal and distal parts and remarks on the regulation of body temperature. Clin. Sci. **3:** 119.

10. Bigelow, N., L. L. Bryan, G. H. Cameron, V. J. Ferreri, S. A. Koroljow & G. I. Manus. 1955. Preliminary report on a study of a correlation between emotional reactions and peripheral blood circulation, using a strain gauge plethysmograph. Psychiat. Quart. **29:** 193–202.
11. Graham, D. T. 1955. Cutaneous vascular reactions in Raynaud's disease and in states of hostility, anxiety, and depression. Psychosomatic Med. **17:**(3).
12. Roth, G., J. B. McDonald & C. Sheard. 1944. The effect of smoking cigarettes and of intravenous administration of nicotine on the electrocardiogram, basal metabolic rate, cutaneous temperature, blood pressure and pulse rate of normal persons. J. Am. Med. Assoc. **125:** 761–767.
13. Goetz, R. H. 1942. Smoking and thromboangiitis obliterans. Clin. Proc. Cape Town. Post-grad. Med. Assoc. **1:** 193–203.
14. Weatherby, J. H. 1942. Skin temperature changes caused by smoking and other sympathomimetic stimuli. Am. Heart J. **24:** 17–30.
15. Mulinos, M. G. & I. Shulman. 1940. The effects of cigarette smoking and deep breathing on the peripheral vascular system. Am. J. Med. Sci. **199:** 708–720.
16. Burn, J. H., E. H. Leach, M. J. Rand & J. W. Thompson. 1959. Peripheral effects of nicotine and acetylcholine resembling those of sympathetic stimulation. J. Physiol. London. **148:** 332–352.
17. Moyer, C. A. & W. G. Maddock. 1940. Peripheral vasospasm from tobacco. A.M.A. Arch. Surg. **40:** 277–285.
18. Stewart, H. J., H. S. Haskell & H. Brown. 1945. The effect of smoking cigarettes on the peripheral blood flow in subjects in the older age group with coronary arteriosclerosis and hypertension. Am. Heart J. **30:** 541–550.
19. Friedell, M. T. 1953. Effect of cigarette smoke on the peripheral vascular system. J. Am. Med. Assoc. **152:** 897–900.
20. Shepherd, J. T. 1951. Effect of cigarette smoking on blood flow through the hand. Brit. Med. J. **2:** 1007–1010.
21. Harpuder, K., I. D. Stein & J. Byer. 1940. The role of the arteriovenous anastomosis in peripheral vascular disease. Am. Heart J. **20:** 539–545.
22. Johnson, H. & J. J. Short. 1933–1934. Effect of smoking on skin temperature. J. Lab. Clin. Med. **19:** 962–966.

INFLUENCE OF NICOTINE ON THE BLOOD FLOW OF RESTING SKELETAL MUSCLE AND OF THE DIGITS IN NORMAL SUBJECTS*

Hans Rottenstein,† George Peirce, Ellier Russ,
David Felder, Hugh Montgomery
Vascular Section, Robinette Foundation, Medical Clinic, Hospital of the University of Pennsylvania, Philadelphia, Pa.

Vasoconstriction in the human hand due to smoking was demonstrated in 1909.[1] Since then numerous studies have amply demonstrated that smoking causes cutaneous vasoconstriction. Intravenous injection of nicotine has been demonstrated to produce cutaneous vasoconstriction,[2] and it is generally accepted that the important vasoactive ingredient in tobacco is nicotine.

Cutaneous vasoconstriction following smoking or injection of nicotine has been determined by skin temperature measurement,[2,3,4] observation of capillary beds,[5] and plethysmograph.[1] An excellent brief summary of early work on cutaneous vasoconstriction caused by nicotine and smoking is given by Roth and associates.[2]

The literature gives somewhat conflicting statements on the effect of nicotine on blood flow in skeletal muscle. In the cat, intra-arterial injection produced both vasoconstriction and vasodilatation, very small doses causing vasodilatation, whereas larger doses resulted first in vasodilatation and then in vasoconstriction.[6] In 1939 studies of the effect of smoking on the blood flow of the hand and forearm were used to provide a reasonable hypothesis that the smoking either produced no change in the flow in the muscles of the forearm, or an increase in the flow.[7] This conclusion is based on the assumption that the blood flow in the skin of the forearm was similar to that in the hand, which is probably true but was not demonstrated.

Further light was thrown on the subject in 1955 by Ruef and his associates[8] who, by use of the Hensel thermoelectric needle, were able to demonstrate by measurements in the muscle that smoking produced a moderate increase in the blood flow in human skeletal muscle.[9]

Because of lack of quantitative data on the effect of smoking on skeletal muscle blood flow and no data on the effect of intravenously administered nicotine on blood flow in human skeletal muscle, it was felt that further studies were necessary, and that these studies should provide a clear distinction between changes in flow in muscle and in skin.

Methods and Procedure

Forty-seven plethysmographic studies and 8 digital temperature studies were made on normal male subjects between the ages of 18 and 31 years. Initially both smokers and nonsmokers were used, but it was found that nonsmokers did not make satisfactory subjects because of frequent attacks of nausea,

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† Presently Anesthesiologist to Carlisle Hospital, Carlisle, Pa.

which caused tensing of muscle incompatible with reliable blood flow measurements.

The nicotine used initially was pure nicotine alkaloid. In later studies nicotine bitartrate solution, supplied by the Tobacco Industry Research Committee, was used. In all cases, doses are reported in terms of the nicotine alkaloid administered.

In nearly all the experiments the intravenous injection of nicotine produced a sensation of heat or burning at the point of injection. Most of the subjects also felt sensations varying from dizziness to sleepiness, numbness of the hands and forehead, and shortness of breath. Nonsmokers tended to become nauseated and two of these vomited. The cigarettes used were the smokers' choices of several common standard brands, without filters.

In preliminary experiments conducted for the purpose of establishing dosages in subsequent studies, varying doses of nicotine alkaloid were injected intravenously at varying rates, using both single and multiple doses in smokers and nonsmokers. Eight subjects were studied. The subject lay on a bed, with toes exposed, in a 20° C. room. Thermocouples were attached to the toes, a slow intravenous drip of 5 per cent dextrose in water was started and, after the temperature of the toes became stable, nicotine was injected into the intravenous drip. Resultant changes in toe temperature were recorded with a Minneapolis-Honeywell potentiometer.

Plethysmographic measurements were not made in a 20° C. room. In these studies blood flow in the muscle of the left calf was measured before, during, and after intravenous nicotine injection or smoking. The muscle plethysmograph was air-filled (FIGURE 1). It consisted of a flanged rubber sleeve, tailored to fit the subject's leg, and an outer plexiglass jacket. An air seal was made between the rubber sleeve and the jacket by means of spring clamps that held the flanges of the rubber sleeve tightly between the ends of the outer jacket and the plexiglass rings.

Changes in limb volume were measured by recording the resultant slight changes in pressure in the plethysmograph by means of a Lilly-Technitrol manometer and a very low pressure transducer constructed in our laboratory. The full-scale sensitivity was about 1 mm. Hg. Calibrating volumes of air were inserted into the system by means of a 5-ml. hypodermic syringe.

Before application of the plethysmograph, the skin of the left calf was iontophoresed with epinephrine to eliminate the effects of skin blood flow.[10] The epinephrine was used at a concentration of 1:1000 and a *p*H of 4.5. Iontophoresis was performed for 20 min. using a current of 30 ma. A profound and lasting vasoconstriction of the skin was produced. This was demonstrated by immersing the limb in warm water subsequent to the experiments; there was erythema in the surrounding area but the iontophoresed area remained blanched.

Following iontophoresis, the subject reclined with head slightly elevated on a pillow and the plethysmograph and a proximal pneumatic cuff, to produce venous occlusions, were attached to the left leg. Each time a plethysmographic measurement was made, this cuff was inflated from a small reservoir at 70 mm. Hg pressure, which produced cuff pressures of 55 to 60 mm. Hg. An arterial pneumatic cuff around the ankle was used to prevent return flow from the foot into the plethysmograph. The use of this arterial cuff limited the permissible length of a series of blood flow measurements to about 20 min. A stethoscope was attached over the left brachial artery, a blood pressure cuff was placed about the left arm, and an intravenous drip of 5 per cent dextrose in water was started in the right arm. The cuff on the left ankle was inflated to 180 mm. Hg, a series of plethysmographic measurements was started and, while they continued, 6 μg. of epinephrine were injected over 15 sec. via the intravenous drip of 5 per cent glucose. Usually blood pressure and pulse and, less frequently, respiratory rates were determined at 1-min. intervals before, during, and after all drug injections.

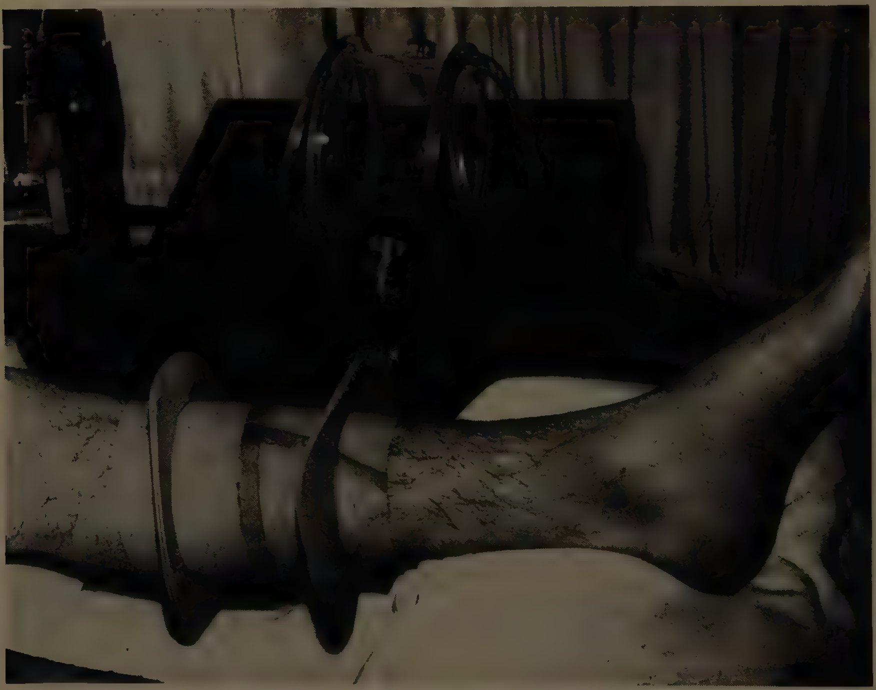


FIGURE 1. Muscle plethysmograph disassembled, showing inner rubber sleeve in place on leg, body of plethysmograph, and plexiglass jacket with clamping rings. The spring clamps are not shown.

When the flow increase had subsided, more plethysmographic measurements were made and the ankle cuff was then deflated. After a recovery period of 10 to 15 min., the ankle cuff was reinflated, a new series of measurements was started and, after 10 to 13 venous occlusions, nicotine alkaloid was injected slowly via the intravenous drip. Measurements were continued for a period of about 10 min. and the ankle cuff was deflated. After another recovery period and reinflation of the ankle cuff, a final series of control measurements was made.

This procedure was followed in 5 experiments in nonsmokers, in which 0.4 to 1.0 mg. of nicotine was injected during 1 min. The procedure was also followed in 18 experiments on smokers, in which 1 to 2 mg. of nicotine were injected during a period of 1 min., and in 16 experiments on smokers in which 3 mg. of nicotine were injected during 4 min.

In 20 of the 1-min. nicotine studies attempts were made to measure simultaneously the blood flow in the contralateral, or right, calf by use of the Hensel thermoelectric needle.

The Hensel thermoelectric needle[8] consists of a 19-gauge hypodermic needle in which is mounted a pair of thermocouples connected in series opposition, so that differential temperatures are measured. One thermocouple is at the tip of the needle, the other 1 cm. from the tip and, at the tip of the needle, there is also a heating element used to supply heat to the end of the needle enclosing the distal thermocouple. When heat was required an alternating current at 1000 cycles was supplied by a stable oscillator. The differential thermocouple output was recorded on a Minneapolis-Honeywell recording potentiometer with a full-scale sensitivity of 10° C.

After an intracutaneous injection of procaine the Hensel needle, with heating current off, was inserted into the right gastrocnemius muscle. After the differential temperature of the needle became steady, a pneumatic cuff about the right thigh was inflated to 180 mm. Hg for 3 min. and then released. If an appreciable change in reading occurred this was considered an artifact, and the needle was moved slightly; if not the distal thermocouple in the needle was heated 4° to 5° C. above the proximal thermocouple and the occlusion and release were repeated. If a satisfactory response (0.3° C. or better) was not obtained on release of the second occlusion, the needle was again moved slightly and the occlusion and release repeated, without and with heating. Results from the needle were used only when the reactive hyperemia produced by release of a 3-min. arterial occlusion resulted in a differential temperature change in the heated needle of 0.3° C. or more, and when the intravenous injection of 6 μg. of epinephrine over 15 sec. (a procedure that invariably produces a great increase in blood flow in muscle) produced a differential temperature change of at least 0.1° C. If after 5 or 6 shifts in the position of the needle a sensitive location could not be found, the plethysmograph alone was used. In 20 experiments in which the needle was used, sufficiently sensitive locations were obtained in only 4.

In 3 of the 1-min. nicotine studies on smokers, and in 3 of the 4-min. nicotine studies on smokers, simultaneous records were made of the blood flow in one of the digits of the right hand using a Cambridge Burch-Winsor plethysmograph. In these experiments the venous occluding cuff at the base of the finger was connected to the pneumatic system for venous occlusions for the leg plethysmograph; this insured simultaneous venous occlusions.

In a set of 8 experiments on 8 subjects in which the leg plethysmograph and epinephrine iontophoresis were employed, but all drug injections and the intravenous drip were omitted, 5 series of 13 occlusions were run with recovery periods of 15 to 20 min. In each experiment, at the end of the thirteenth occlusion of the fourth series of occlusions, a cigarette was smoked at the subject's usual rate. Occlusions were continued while the subject was smoking.

Results

(1) In a series of 8 experiments on 8 subjects in a 20° C. room, the injection of 1 to 2 mg. of nicotine caused a reduction of toe temperature in 7 of the subjects, commencing 1 to 2 min. after the injection. In one subject there was no change. In another of the subjects the temperature drop was followed by a sudden rise to a temperature higher than that measured before the injection. This effect coincided approximately with the onset of nausea in the subject.

TABLE 1A

MEAN AND PEAK RESPONSES OF SMOKERS TO ONE-MINUTE NICOTINE INJECTIONS*

No.	Dose (mg.)	Muscle blood flow: Ml./100 ml./min. Control	Muscle blood flow: Ml./100 ml./min. 60–260 sec. after inj.	Muscle blood flow: Change (%)	Muscle blood flow: Peak rise (%)	Muscle blood flow: Time of peak† (sec.)	Blood pressure: Syst. change (%)	Blood pressure: Diast. change (%)	Pulse change (%)	Resp. change (%)
1A	2.0	2.86	5.76	+101	176	66	+10	+5	+81	—
6	1.5	3.24	4.43	+37	98	154	—	—	—	—
8A	1.5	2.63	3.84	+46	147	112	—	—	—	—
8B	2.0	2.39	3.61	+65	109	166	—	—	—	—
9	2.0	2.10	2.72	+29	105	57	+10	+16	+27	—
10A	2.0	2.87	4.61	+61	221	123	+3	−1	0	—
11	2.0	2.45	4.58	+87	153	134	+19	+4	+37	+41
12A	1.0	3.32	4.87	+47	75	153	+4	+6	+10	+14
13A	1.0	2.22	2.98	+34	57	157	+1	−2	−1	—
10B	2.0	2.37	2.72	+15	60	227	+8	+8	+6	—
8C	2.0	3.12	3.39	+9	70	140	+14	+10	+13	0
13B	2.0	2.35	3.19	+36	66	107	+14	+6	+5	—
14A	2.0	2.61	4.34	+66	191	143	+9	0	+6	+80
15	2.0	2.26	2.63	+16	59	107	+6	−8	+11	—
17	2.0	2.36	3.88	+64	188	109	+5	+1	+26	—
8D	2.0	2.78	6.32	+127	353	120	+13	+7	+15	−4
18	2.0	3.73	6.47	+100	340	141	+5	+14	+36	+22
19A	2.0	1.51	2.01	+33	85	127	+10	+8	+8	+33
Mean		2.62	4.02	+54 $P < 0.01$	142	130	+9 $P < 0.01$	+5	+19 $P < 0.01$	+27

* No. refers to subject. Number 1A refers to the first use of subject 1, and 6 refers to the only use of subject 6. Percentages of changes in blood pressure, pulse, and respiration are derived from the mean of the measurements made during the same period after the injection of nicotine used for deriving the blood-flow changes. Peak use refers to highest flow measured after nicotine injection.

† Occasionally there were several readings with equal highest flow, in which case the time of the first is given.

(2) The injection of 6 μg. of epinephrine during 15 sec. always produced a large transitory increase in the blood flow in skeletal muscle, as measured by the leg plethysmograph. The peak increases observed varied from 110 per cent to 1017 per cent of the mean flow before injection, with a mean peak rise of 522 per cent.

(3) The changes in muscle flow caused by intravenous nicotine and measured by the plethysmograph are shown in TABLES 1A, 1B, and 1C. The intravenous injections of 1 to 2 mg. of nicotine administered during 1 min. caused an increase

in mean blood flow during the period 60 to 260 sec. after the start of the injection (TABLE 1A). Increase in blood flow is significant at the 0.01 level for this series. Intravenous injections of from 0.4 to 1 mg. of nicotine over 1 min. to nonsmokers in all cases caused an increase in mean blood flow in muscle during the period 60 to 260 sec. after the injection (TABLE 1B). The result is signifi-

TABLE 1B
MEAN AND PEAK RESPONSES OF NONSMOKERS TO ONE-MINUTE NICOTINE INJECTIONS*

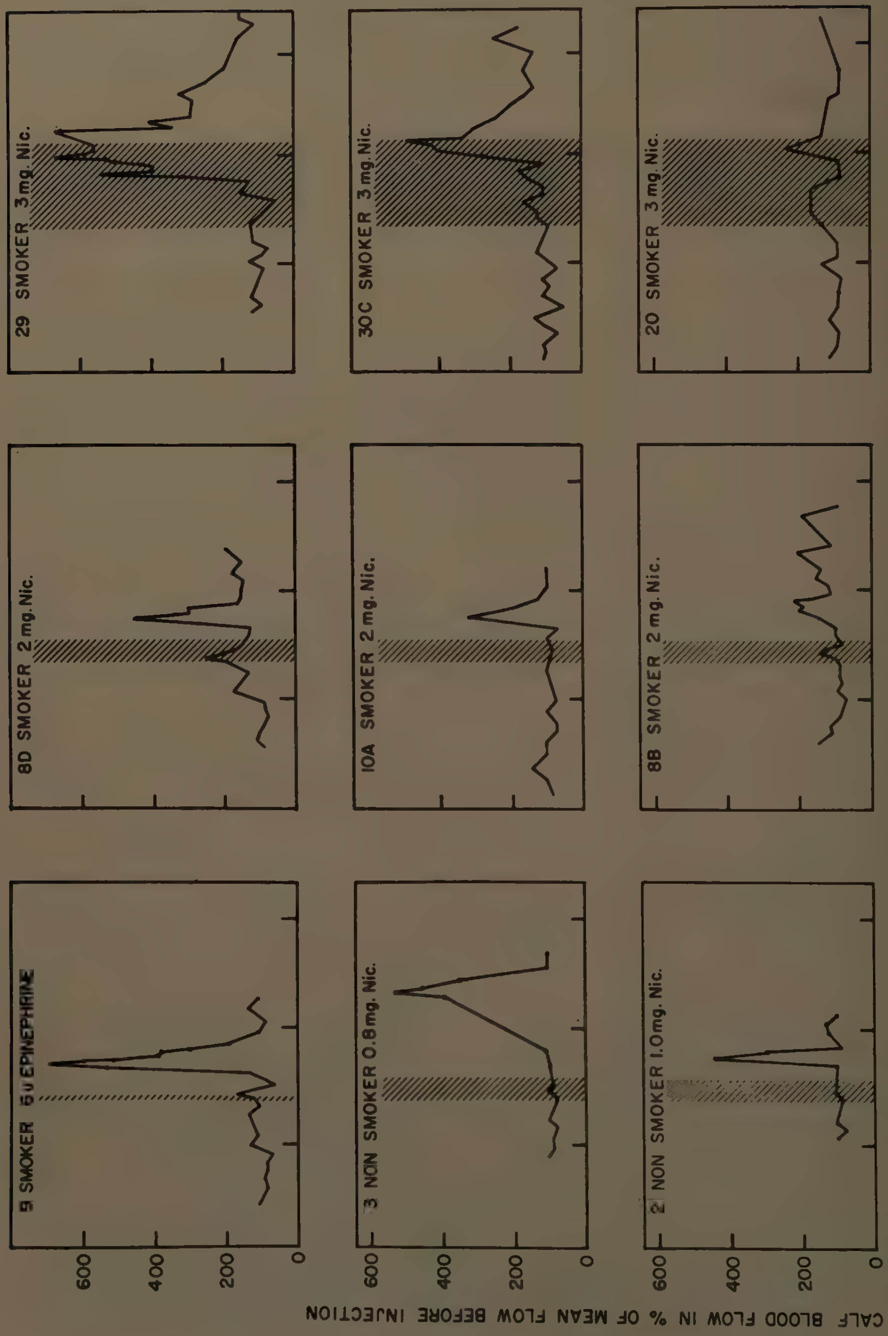
No.	Dose (mg.)	Muscle blood flow				
		Ml./100 ml./min.		Change (%)	Peak rise (%)	Time of peak (sec.)
		Contro	60–260 sec. after inj.			
2	1.0	1.20	2.24	+87	342	123
3	0.8	1.84	3.80	+107	538	296
4	0.4	3.93	6.76	+72	195	185
5	0.6	2.50	2.94	+118	52	338
7	1.0	2.20	3.27	+49	217	365
Mean		2.33	3.80	+88 P < 0.05	269	261

* See footnote to TABLE 1A.

TABLE 1C
MEAN AND PEAK RESPONSES OF SMOKERS TO FOUR-MINUTE NICOTINE INJECTIONS*

No.	Dose (mg.)	Muscle blood flow					Blood pressure		Pulse change (%)	Resp. change (%)
		Ml./100 ml./min.		Change (%)	Peak rise (%)	Time of peak (sec.)	Syst. change (%)	Diast. change (%)		
		Control	120–360 sec. after inj.							
1B	3.0	2.53	6.65	+163	362	159	—	—	—	—
20	3.0	2.33	3.54	+30	127	210	0	−6	+4	+8
21	3.0	3.02	5.73	+90	148	271	+6	+26	+6	+30
22	3.0	1.58	4.43	+174	318	267	+19	+8	+6	+23
23A	3.0	3.20	3.71	+16	56	146	+7	−1	+5	+5
24	3.0	1.55	2.52	+63	107	104	+19	+9	+27	+20
25	3.0	2.11	1.79	−15	4	257	+4	−6	+17	+5
26	3.0	2.04	2.18	+7	106	510	+26	+24	+16	+10
27A	3.0	1.48	2.99	+102	224	253	+3	+9	+22	+35
28	3.0	1.41	1.67	+18	66	55	+28	+16	+1	+24
29	3.0	1.59	3.49	+119	561	266	+15	+14	+17	+25
30C	3.0	2.16	5.87	+172	386	233	+16	+3	+31	—
23B	3.0	1.39	1.31	−6	23	418	+6	+13	+5	+15
14B	3.0	0.90	1.80	+100	189	323	+14	+9	+10	+40
12B	3.0	1.44	2.27	+58	136	382	+6	+25	−3	+20
32	3.0	1:39	2.57	+85	181	138	+5	+7	+23	+20
Mean		1.88	3.28	+73 P < 0.01	187	250	+12 P < 0.01	+10	+12 P < 0.01	+20 P < 0.01

* See footnote to TABLE 1A.


29 SMOKER 3mg.Nic.
30C SMOKER 3mg.Nic.
20 SMOKER 3 mg.Nic.
8D SMOKER 2 mg.Nic.
10A SMOKER 2 mg.Nic.
8B SMOKER 2 mg.Nic.
9 SMOKER 6u EPINEPHRINE
3 NON SMOKER 0.8mg. Nic.
2 NON SMOKER 1.0mg. Nic.
600
400
200
0
CALF BLOOD FLOW IN % OF MEAN FLOW BEFORE INJECTION

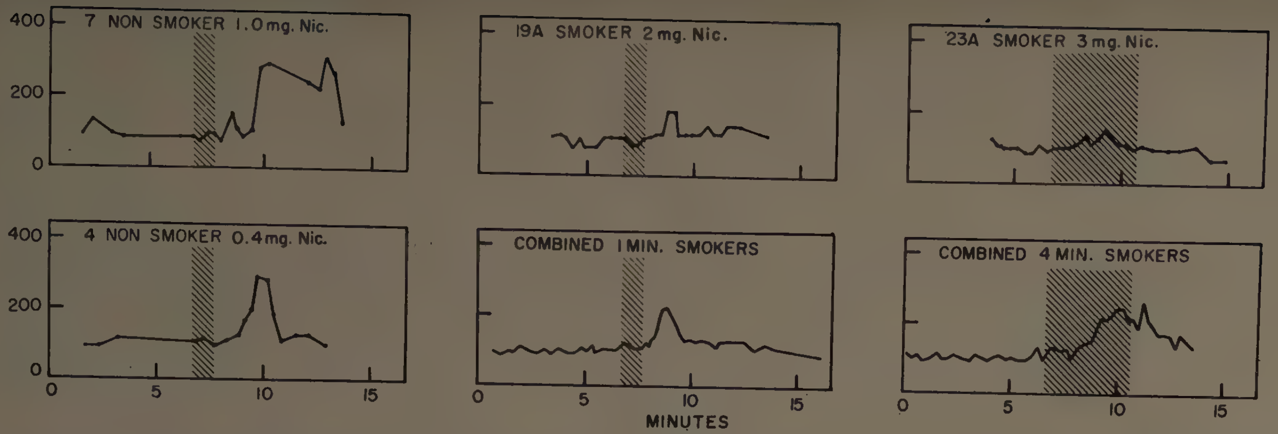


FIGURE 2. Plethysmographic measurements of muscle blood flow changes produced by nicotine and epinephrine. Shaded area represents period of drug injection, which was 15 sec., 1 min., or 4 min. Drug dose is shown below the upper border of each block except in the lowest block in the middle column, which shows the combined result of all experiments in which nicotine was injected over 1 min., and the lowest block in the right-hand column, which shows the combined result of all experiments in which nicotine was injected over 4 min.

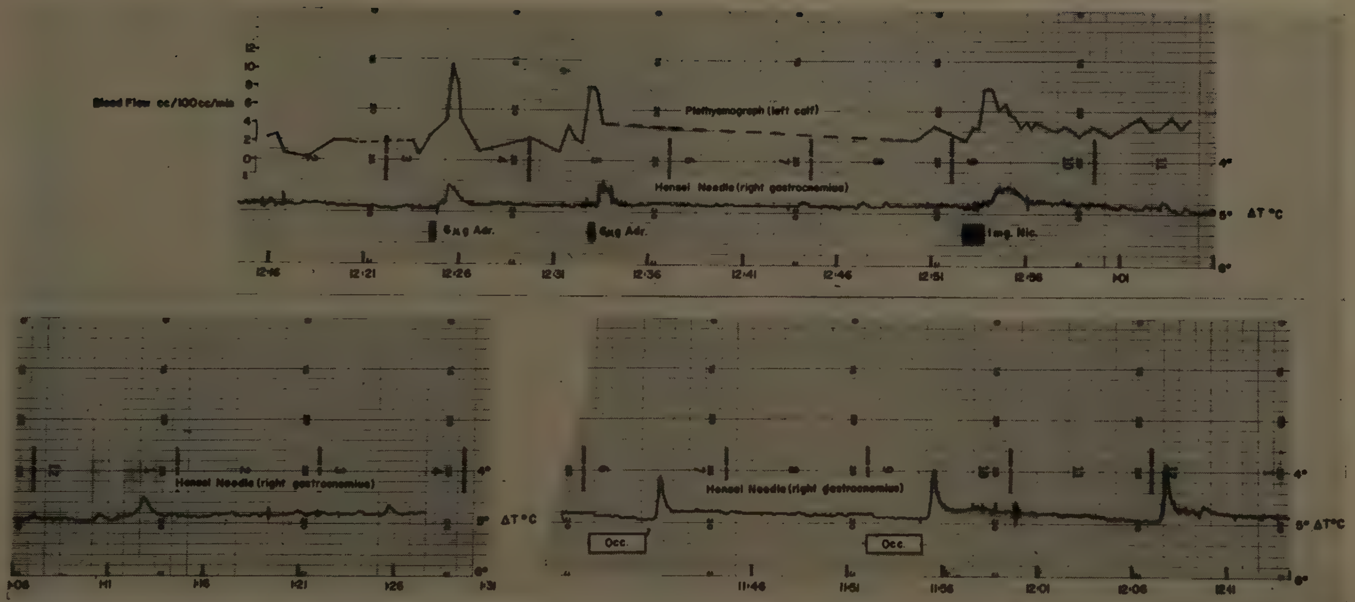


FIGURE 3. Tracing of one continuous record of blood-flow changes by Hensel needle, above part of which is plotted the blood flow measured simultaneously in the contralateral limb by plethysmograph. The top strip shows the responses to 2 injections of epinephrine (Adr.) and to one of nicotine. The lower left strip shows sudden variations in blood flow frequently found in Hensel needle records late following (but rarely before) nicotine injections. The lower right strip shows the response to the release of 3 arterial occlusions (first 2 marked).

cant at the 0.05 level but not at the 0.01 level. The injection of 3 mg. of nicotine over 4 min. to smokers caused an increase in mean muscle flow during the period 2 to 6 min. after the start of the injection (TABLE 1C). The increase is significant at the 0.01 level.

TABLE 2
COMPARISON OF MEAN BLOOD FLOWS IN MUSCLE IN CONTROL RUNS BEFORE AND AFTER DRUG INJECTIONS

No.	Control before epinephrine injection		Control before nicotine injection		Control after nicotine injection	
	Blood flow	Minutes before nicotine injection	Blood flow	Minutes before nicotine injection	Blood flow	Minutes after nicotine injection
One-minute inj. smokers						
1A	2.13	26	2.86	3	4.20	29
6	3.07	54	3.24	3	3.10	27
8A	2.22	42	2.63	5	4.40	47
8B	1.68	37	2.39	2	2.97	33
10A	2.20	29	2.87	3	3.58	47
11	2.54	55	2.45	2	4.20	34
13A	1.89	38	2.22	3	2.72	47
10B	2.49	34	2.37	2	2.66	42
8D	3.28	24	2.78	3	2.76	26
18	3.37	26	3.73	2	4.00	30
19A	1.74	29	1.51	2	1.81	32
Mean	2.42	35.8	2.64	2.7	3.31	35.8
Four-minute inj. smokers						
1B	1.80	29	2.53	2	2.73	28
20	2.64	32	2.33	3	2.25	39
21	3.62	31	3.02	3	3.64	33
23A	3.71	26	3.20	1	2.02	29
24	0.85	60	1.55	2	1.19	26
25	1.90	39	2.11	4	2.43	24
26	2.07	30	2.04	3	2.05	31
27A	1.58	27	1.48	2	1.37	26
28	1.05	30	1.41	3	1.71	17
29	1.21	24	1.59	2	1.71	28
30C	2.12	25	2.16	4	1.89	22
23B	1.56	25	1.39	3	1.98	22
14B	0.85	48	0.90	4	1.01	21
32	1.45	25	1.39	3	2.08	24
Mean	1.89	32.2	1.94	2.8	2.00	26.4

(4) In FIGURE 2 are shown the plots of representative experiments in which the responses to nicotine injection were measured by leg plethysmograph. In the left column the results of 4 experiments on nonsmokers are plotted. The top curve in this column is a plot of a typical response to an injection of 6 μg. of epinephrine. In the center column are shown the responses in 4 experiments on smokers to the injection of 1 to 2 mg. of nicotine over 1 min. The bottom curve in this column is the mean response of the entire group of 18 experiments to 1-min. injections. In the right hand column representative results from the

injection of 3 mg. of nicotine during 4 min. are shown, with the bottom curve expressing the mean response for the entire group of 16 experiments.

(5) In 5 experiments in which a finger plethysmograph was used, as well as a muscle plethysmograph, the digital flow decreased before the muscle flow increased. The digital flow remained at a low level after the large transitory increase in muscle flow was over.

(6) In the 4 cases in which a sensitive location for the Hensel needle was obtained, the needle record showed a transitory increase of blood flow after injection of nicotine, confirming the plethysmographic results and showing beyond doubt that the increase took place in skeletal muscle. FIGURE 3 shows a typical successful simultaneous run, using both needle and leg plethysmograph.

(7) In most of the experiments a final control series of occlusions was run about thirty minutes after the nicotine response was over (TABLE 2). After both the 1-min. and the 4-min. injections the average final flow level was above the flow before injection but not sufficiently high to establish significance for a longer-term effect.

TABLE 3
EFFECT OF CIGARETTE SMOKING ON MUSCLE BLOOD FLOW

No.	First control period		Second control period		Third control period		Fourth control period		While smoking	Fifth control period	
	Mean blood flow	Minutes before smoking	Mean blood flow	Minutes before smoking	Mean blood flow	Minutes before smoking	Mean blood flow	Minutes before smoking	Mean blood flow	Mean blood flow	Minutes after smoking
34	1.78	83	1.93	60	2.20	34	2.35	5	2.21	2.75	30
35	1.81	82	2.08	57	1.71	30	2.70	6	2.22	2.41	27
30A	2.43	56	1.89	37	1.75	22	2.13	6	2.29	2.66	18
30B	2.21	95	2.15	60	1.54	32	2.11	5	2.57	1.70	25
27B	1.50	83	1.86	58	1.98	36	1.93	8	2.44	1.64	21
27C	1.47	56	1.36	41	1.36	22	1.25	7	3.10	1.90	13
36	0.82	69	0.81	58	0.75	34	0.94	8	0.92	0.93	16
37	0.90	85	0.89	73	0.95	56	0.84	19	0.89	1.08	45
Mean	1.62	76.1	1.62	55.5	1.53	33.3	1.78	8.0	2.08	1.88	24.4

(8) The smoking of 1 cigarette in a series of 8 experiments upon 6 subjects caused a rise in the mean blood flow in 5 experiments. The data in TABLE 3 show an increase in mean blood flow, but it would take many more experiments of this type to show a high level of significance.

Summary

The intravenous injection of nicotine produces in most subjects a pronounced transitory increase in the blood flow to muscle and a somewhat more prolonged decrease in the blood flow to the fingers.

Acknowledgments

We thank Abner Eisner for an early supply of pure nicotine alkaloid, and the Tobacco Industry Research Committee for standard solutions of nicotine bitartrate.

References

1. Bruce, J. W., J. R. Miller & D. R. Hooker. 1909. The effect of smoking upon the blood pressures and upon the volume of the hand. Am. J. Physiol. **24:** 104.
2. Roth, G. J., J. B. McDonald & C. Sheard. 1944. The effect of smoking cigarettes and of intravenous injections of nicotine on the electrocardiogram, basal metabolic rate, cutaneous temperature, blood pressure and pulse rate of normal persons. J. Am. Med. Assoc. **125:** 761.
3. Wright, I. S. 1933. Clinical value of human capillary studies in fever, mental deficiency, nephritis, vascular diseases, clubbed fingers, arthritis, tobacco smoking and argyria. J. Am. Med. Assoc. **101:** 439.
4. Maddock, W. G. & F. A. Coller. 1932. Peripheral vasoconstriction by tobacco demonstrated by skin temperature changes. Proc. Soc. Exptl. Biol. Med. **29:** 487.
5. Wright, I. S. & D. Moffat. 1934. The effects of tobacco on the peripheral vascular system. J. Am. Med. Assoc. **103:** 315.
6. Hilton, S. M. 1954. The effects of nicotine on the blood vessels of skeletal muscles in the cat. J. Physiol. **123:** 289.
7. Abramson, D. I., H. Zazella & B. S. Oppenheimer. 1939. Plethysmographic studies of peripheral blood in man. III. Effect of smoking upon the vascular beds in the hand, forearm and foot. Am. Heart J. **18:** 290.
8. Hensel, H. & J. Ruef. 1954. Continuous registration of muscular blood flow in man with calorimeter sound. Arch. ges. Physiol. **259:** 267.
9. Ruef, J., K. D. Bock & H. Hensel. 1955. Ueber die Wirkung des Rauchens auf die Muskeldurchblutung. Z. Kreislaufforschung. **44:** 272.
10. Barcroft, H., W. McK. Bonnar, O. G. Edholm & A. S. Effron. 1943. On sympathetic vasoconstrictor tone in human skeletal muscle. J. Physiol. **102:** 21.

EFFECT OF SMOKING ON THE PERIPHERAL CIRCULATION IN RELATION TO ENVIRONMENTAL TEMPERATURE

J. Edwin Wood

Georgia Heart Association Laboratory for Cardiovascular Research, Department of Medicine, Medical College of Georgia, Augusta, Ga.

The effects of the use of tobacco upon the peripheral blood vessels present numerous problems with great variation in difficulty of assessment. The long-term relationship between smoking and atherosclerosis is one of the most formidable of these problems. However, the pharmacology of nicotine is well described, and the complex but clinically more important acute effects of smoking tobacco upon the circulation of the resting patient have also been studied extensively. The purpose of the experiments described here was to study further the acute effects of smoking on the peripheral circulation of the foot and calf. Special emphasis was placed upon the relationship between the effects of tobacco and the effects of a cool environment in order to obtain at least a qualitative appraisal of the severity of the changes induced by smoking. The problem of whether or not the peripheral blood vessels develop tolerance to the effects of tobacco was investigated. Finally, an attempt was made to evaluate the effect of smoking in a situation other than that of the resting circulation; namely, the effect of smoking upon the reactive hyperemia of a previously ischemic limb.

Methods

Normal subjects in these experiments were young males ranging from 26 to 33 years of age. The subjects for the studies of the effect of abstinence from tobacco were physicians who habitually inhaled tobacco smoke and regularly consumed more than one pack of cigarettes per day. Subjects for study of the effect of smoking upon blood flow in the presence of vascular disease were hospitalized patients. All of the studies were carried out in a constant-temperature room. Temperature was maintained either at 83° F. (warm room) or 68° F. (cool room), with equipment that allowed control of these temperatures within ±1° F. Measurements of blood flow of the foot or of blood flow of the calf were made with the venous occlusion plethysmographic method. Arterial occlusion was applied to the ankle during all measurements of blood flow of the calf. Observations were made when the subject was pursuing his regular smoking habits. Then he discontinued smoking for 24 to 48 hours and the observations were repeated. The tests were performed 2½ to 3 hours after the noon meal. The subjects were studied while wearing shorts, lying supine in a comfortable bed. They were encouraged to relax but were not permitted to sleep, and generally carried on no conversation with the investigators.

Procedure

The subject was exposed to the warm environment for a period of 1 hour, during which time the plethysmograph was applied and filled with water to a temperature of 89° F. As soon as blood flow was reasonably stable over a period of 15 min. the subject smoked two cigarettes at his own rate. Low nicotine, "king" size, or filter tip cigarettes were not used. The observations were continued at brief intervals for a period of 30 to 60 min.

Studies of the effect of smoking upon reactive hyperemia blood flow were carried out by occluding the circulation to the foot or calf, after 5 min. of smoking, for a further 5-min. period. After this the circulation was restored and blood flow was measured at 15-sec. intervals for 2 min., and then at 30-sec. intervals for the next 6 min. as smoking continued. The same experiment had been carried out earlier, but without smoking.

Blood flow values are given as ml./min./100 ml. of foot tissue or of calf tissue. Data on the studies of reactive hyperemia are given in terms of the total quantity of blood that flowed through the foot during the 2-min. period following release of the circulation in ml./100 ml. of foot tissue or of calf tissue.

Results

Most of these data have been presented in detail elsewhere,[1,2] and they are given only in outline here. Thirty-one experiments were performed on the effect of smoking two cigarettes at room temperatures of 83° F. and 68° F. upon the resting blood flow of the foot. In all but 3 of these experiments the average blood flow decreased during smoking. Even in these experiments the blood pressure and pulse rate increased, and the variability of blood flow diminished. In the warm environment the vasoconstriction persisted for 20 to 40 min., while in the cool room it lasted from 20 to 50 min. Changes in the skin temperature of the toe were consistent with the changes in blood flow, but tended to lag behind the changes of flow.

In 17 experiments in the warm environment blood flow decreased an average of 1.5 ml./min./100 ml., while in 14 experiments in the cool room the blood flow reduction averaged 0.3 ml./min./100 ml. There was a marked difference in the range of absolute reduction of blood flow, but the percentage reduction of blood flow was about the same in either case. In 13 paired experiments the reduction of blood flow with smoking in the warm room averaged 20 per cent and in the cool room averaged 22 per cent.

The effect of abstinence from tobacco. Experiments on 11 subjects before and after abstinence from tobacco showed no significant difference in the vasoconstrictor effects of smoking. However, most of the subjects reported symptoms of dizziness or even nausea when smoking for the first time after abstinence.

The effect of change in room temperature. In 13 experiments on the effect of smoking in the warm environment and the effect of cooling the environment, reduction of blood flow by cooling was greater than that of smoking in the warm environment in all but 2 of the experiments. Smoking in the cool environment did, however, reduce blood flow further as noted above.

The effect of smoking upon reactive hyperemia blood flow. In 27 experiments in the warm environment the blood flow during reactive hyperemia was reduced beyond that expected from random variation on 15 occasions. Average blood flow in the control period during reactive hyperemia was 18 ml./100 ml. of foot tissue and averaged 13.7 ml./100 ml. of foot tissue during smoking in all subjects.

In 14 experiments in the cool environment smoking produced a decrease in reactive hyperemia blood flow in 6 studies. In the cool environment reactive hyperemia blood flow averaged 13.6 ml. in the control period and averaged 9.9 ml. during smoking in all subjects.

The effect of a cool environment upon the blood flow during reactive hyperemia. Fourteen of 17 subjects showed a definite reduction of reactive hyperemia blood flow when the room temperature was reduced to 68° F. without smoking. The average reactive hyperemia blood flow in the warm environment was 19.9 ml., while it averaged 12.6 ml. in the cool environment in all subjects.

The effect of sympathectomy upon the reduction of blood flow during reactive hyperemia. Four patients with unilateral sympathectomies of the lower extremities were studied, and it was found that reduction of reactive hyperemia blood flow by smoking could be observed in the unsympathectomized limbs in both the warm and cool environment, but not observed in the sympathectomized limbs in either environment.

The effect of vasospastic peripheral vascular disease. Fourteen of the subjects were considered to have peripheral vascular disease that was vasospastic in nature, but there was no apparent exaggeration of the vasoconstrictor responses induced by smoking in these subjects.

The effect of smoking in the warm environment upon resting flow and reactive hyperemia flow of the calf was studied in 6 subjects. There was no consistent effect of smoking upon the blood flow of the calf with smoking, either at rest or during reactive hyperemia.

Discussion

The effects of smoking tobacco upon the peripheral circulation, particularly with reference to blood flow, have been extensively investigated. Most studies, although not all, indicate that the inhalation of tobacco smoke reduces the blood flow of the skin, especially of the toes. Although this relatively general agreement existed as to the direction of change of blood flow with smoking, it seemed reasonable to further evaluate the effects of smoking by comparing its effect with a mild vasoconstrictor stimulus of a cool environment of 68° F. The reduction of resting blood flow of the foot by smoking in a warm environment was less than that produced by cooling the environment. The results of these experiments, as well as of others, leave little doubt as to the direction of change of blood flow induced by smoking and are commensurate with the known effects of small doses of nicotine. When smoking is compared with a relatively usual everyday activity, namely, exposure to a moderately cool environment, the vasoconstrictor effects would seem no more hazardous than those to which the individual is already exposed. It is important to point out, on the contrary, that a marked reduction of blood flow induced by a cool environment may be accompanied by a further reduction of flow with the additional stimulus of smoking. Thus if the circulation to the skin is greatly compromised, mild cold or smoking might be detrimental.

Since some patients smoke only occasionally the question arises as to whether they would ordinarily develop tolerance to tobacco, causing much slighter effects upon the circulation than when tobacco is used almost continuously. Experiments described here indicate that abstinence from tobacco does not result in an accentuation of the vasoconstrictor responses of the foot to smoking.

Thus the patient need not fear an accentuation of the vasoconstrictor effects of tobacco by intermittent smoking.

An attempt was made to assess the effect of smoking tobacco upon blood flow that might not be so redundant as that of the resting circulation, that is, of blood flow passing through the foot during a period of reactive hyperemia. The proportion of the blood flowing through capillaries of the foot following a period of ischemia, relative to that flowing through arteriovenous shunts, cannot be evaluated by methods utilized in these studies. It seems reasonable to assume, however, that there is a metabolic need for a larger proportion of the blood flow than in the resting situation. In any event, smoking tobacco does depress the amount of blood that passes through the foot during reactive hyperemia, as does a cool environment. The combination of smoking and of cooling depresses reactive hyperemia further. These studies also suggest that in situations of a critical ratio between the need for blood and availability of blood, the factors that cause reactive hyperemia[3] might be of greatest importance; smoking, or smoking in combination with a cool environment, might be detrimental to the patient. On the other hand, as shown above, the effect of smoking upon reactive hyperemia is if anything less than that produced by a cool environment alone.

The effects of smoking upon resting blood flow and upon the reactive hyperemia of the foot undoubtedly represent the effects of this procedure upon the circulation of the skin. Studies of the effects of these same procedures upon the blood flow of the calf indicated that no such responses occurred. The calf is composed primarily of skeletal muscle, while the foot is primarily skin. Numerous studies of the circulation of muscle versus those of skin have indicated that the responses of these vascular beds are fundamentally different.[4] The studies described herein would lead to the conclusion that a patient who has diminished blood flow to the calf, with intermittent claudication, could not reasonably expect an improvement in his situation if he stopped smoking.

Studies of patients with sympathectomized limbs indicate that the effect of smoking upon the blood flow of the foot is mediated primarily via the sympathetic nervous system rather than through the release of catecholamines[5] from other sites or the direct effects of nicotine upon the smooth muscle of the blood vessels themselves. Furthermore, these studies suggest that patients who have critically low blood flow in the skin and who might suffer further damage with smoking[6] would be protected by sympathectomy.

Summary and Conclusions

(1) Smoking tobacco causes vasoconstriction and decrease in blood flow of the foot.

(2) Smoking depresses the blood flow of the foot (primarily skin) during reactive hyperemia.

(3) A cool environment of 68° F. produces a greater reduction of resting blood flow and of reactive hyperemia blood flow of the foot than does smoking.

(4) The vasoconstrictor effects of smoking and of a cool environment on the circulation of the foot are not observed in sympathectomized limbs.

(5) These vasoconstrictor effects of smoking are not observed in the calf.

(6) Possible clinical implications of these findings are discussed.

References

1. Eckstein, J. W., J. E. Wood & R. W. Wilkins. 1957. Comparative vasoconstrictor effects of inhaling tobacco smoke in warm and cool environments and before and after abstinence from tobacco. Am. Heart J. **53:** 455.
2. Coffman, J. D., J. E. Wood & R. W. Wilkins. 1958. Effect of cooling and of smoking tobacco upon the blood flow of reactive hyperemia of the foot. Circulation. **28**: 177.
3. Wood, J. E., J. Litter & R. W. Wilkins. 1955. The mechanism of limb segment reactive hyperemia in man. Circulation Research. **3:** 581.
4. Barcroft, H. & H. J. C. Swan. 1953. Sympathetic Control of Human Blood Vessels. Arnold. London, England.
5. Rehder, K. & G. M. Roth. 1959. Effect of smoking on the fasting blood sugar and pressor amines. Circulation. **20:** 224.
6. Juergens, J. L., N. W. Barker & E. A. Hines, Jr. 1960. Arteriosclerosis obliterans: Review of 520 cases with special reference to pathogenic and prognostic factors. Circulation. **21:** 188.

THE ACUTE EFFECT OF CHEWING TOBACCO AND SMOKING IN HABITUAL USERS*

David L. Simon and Arnold Iglauer

The Cardiac Laboratory of the Cincinnati General Hospital, University of Cincinnati College of Medicine, Cincinnati, Ohio

The circulatory effects of tobacco were studied in 14 young male cigarette smokers without cardiovascular disease,[1] 24 habitual users of chewing tobacco,[2] and 25 cigar and pipe smokers[3] following the use of these various forms of tobacco. The same methods of study were used in all observations of smokers and chewers.

Studies were carried out in the basal postabsorptive state on each subject on three separate days. On one day low-nicotine tobacco was smoked in a constant temperature room, and on another day regular tobacco was smoked. On a third day the effect of high-nicotine tobacco on the ballistocardiogram was determined; the order of studies was variable. Skin temperatures were measured in a constant temperature room at 78° F. (25.6° C.) and 40 per cent relative humidity from copper-constantan thermocouples attached to the forehead and the volar surfaces of the fingers and toes, employing a Brown constant-recording potentiometer. Changes of 0.5° C. were considered significant. Blood pressures and pulses were obtained by the usual clinical method. The ballistocardiograms were recorded with a high-frequency table-type research instrument.[4] The subjects were supine and unclothed and rested for at least 45 min. prior to smoking or chewing. The period of smoking or chewing was 20 min.

The young cigarette smokers were 14 convalescent male patients, without cardiovascular disease, from the surgical service of the Cincinnati General Hospital and three normal male volunteers, all of whom were habitual smokers. Their ages ranged between 16 and 41 (average age—27.2). Each subject smoked two low-nicotine cigarettes through a silica filter on the first day and one or two ordinary cigarettes, with no filter, on the second and third day.

The tobacco chewers were 24 male volunteers who chewed tobacco habitually; their ages ranged between 34 and 71 years (average age—51.1). Each subject chewed, on alternate days, a full mouthful of low-nicotine chewing tobacco (0.31 to 0.47 per cent nicotine) and a regular commercial brand (1.53 per cent nicotine). Chewing gum was used in several subjects as a placebo. The average amount of tobacco chewed weighed 10 gm.

The cigar and pipe smokers were 25 habitual male smokers; their ages ranged between 21 and 69 years. Eight subjects were studied while sham-smoking unlighted pipes or cigars under identical conditions. The commercial cigars were a well-known brand containing 1.82 per cent nicotine (moisture-free basis). The low-nicotine cigars were a commercially available brand containing 0.88 per cent nicotine. The pipe tobacco used was a commercial brand containing 2.15 per cent nicotine (moisture-free basis). Pipe tobacco containing 0.72 per cent nicotine was used for the low-nicotine studies.

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The low-nicotine cigars weighed 6.6 gm.; the regular cigars weighed 6.5 gm. A pipeful of tobacco weighs approximately 2.0 gm. By comparison, a standard cigarette contains 1 gm. of tobacco and approximately 20 mg. (2 per cent) nicotine.

Results

CIGARETTE SMOKERS

No change in the form of ballistocardiograms occurred after smoking either low-nicotine filtered or ordinary cigarettes; cardiac output computed from ballistocardiograms did not change.

When the pulse rates were compared statistically before and after the smoking of both the low-nicotine filtered and ordinary cigarettes, no significant difference was found with the low-nicotine cigarettes, but a statistically significant increase in pulse rate occurred after the smoking of standard cigarettes ($t = 3.0$, $P < 0.01$).

In general the skin temperature changes in all our subjects followed the well-known pattern already previously described by Grollman,[5] Roth,[6] and others,[7,8] although in our subjects the temperature fall was not as pronounced in the lower extremities.

CHEWERS

Ballistocardiograms. After the chewing of standard commercial tobacco, ballistocardiograms of 23 of the 24 subjects showed changes of the ballistic wave pattern from the control pattern taken before they had begun chewing. These changes were usually maximal 15 min. after chewing began, and the patterns gradually returned to normal. The changes were generally of the early M pattern. In the patient whose ballistocardiogram is shown in FIGURE 1, no changes were seen 15 min. after chewing the placebo gum (FIGURE 2).

Pulse rate. After the subjects chewed commercial tobacco, the pulse rate of 1 remained constant and the pulse rates of 14 increased markedly (FIGURES 3, 4). The range of increase was from 1 to 24 beats/min., with an average increase of 13.4 beats/min. With low-nicotine tobacco, the pulse rate remained constant in three subjects, decreased in 1, and increased in 9 (range of increase: 2 to 10; mean: 6.5 beats/min.). With gum, 1 subject showed a slight rise and 1 showed a slight fall in pulse rate.

Blood pressure. The blood pressures of 16 subjects were measured; all showed a rise in systolic pressure after chewing commercial tobacco, and 15 showed a rise in diastolic pressure. The range of rise in systolic pressure was 10 to 45 mm. Hg with a mean rise of 17.9 mm. Hg. The range of rise in diastolic pressure was 5 to 20 mm. Hg, with a mean of 11.8 mm. Hg. In 1 subject whose blood pressure rose 15/15 mm. Hg after he chewed commercial tobacco, no rise was recorded after chewing gum (FIGURE 5).

Skin temperatures. In most cases the pattern of temperature changes following the chewing of tobacco was similar to that previously reported in connection with cigarette smoking. Forehead temperatures remained almost constant at 35° C. (95° F.) with both types of tobacco and with gum. Initial toe temperatures ranged from 23 to 35° C. (73.4° to 95° F.), with a mean of 29.7° C.

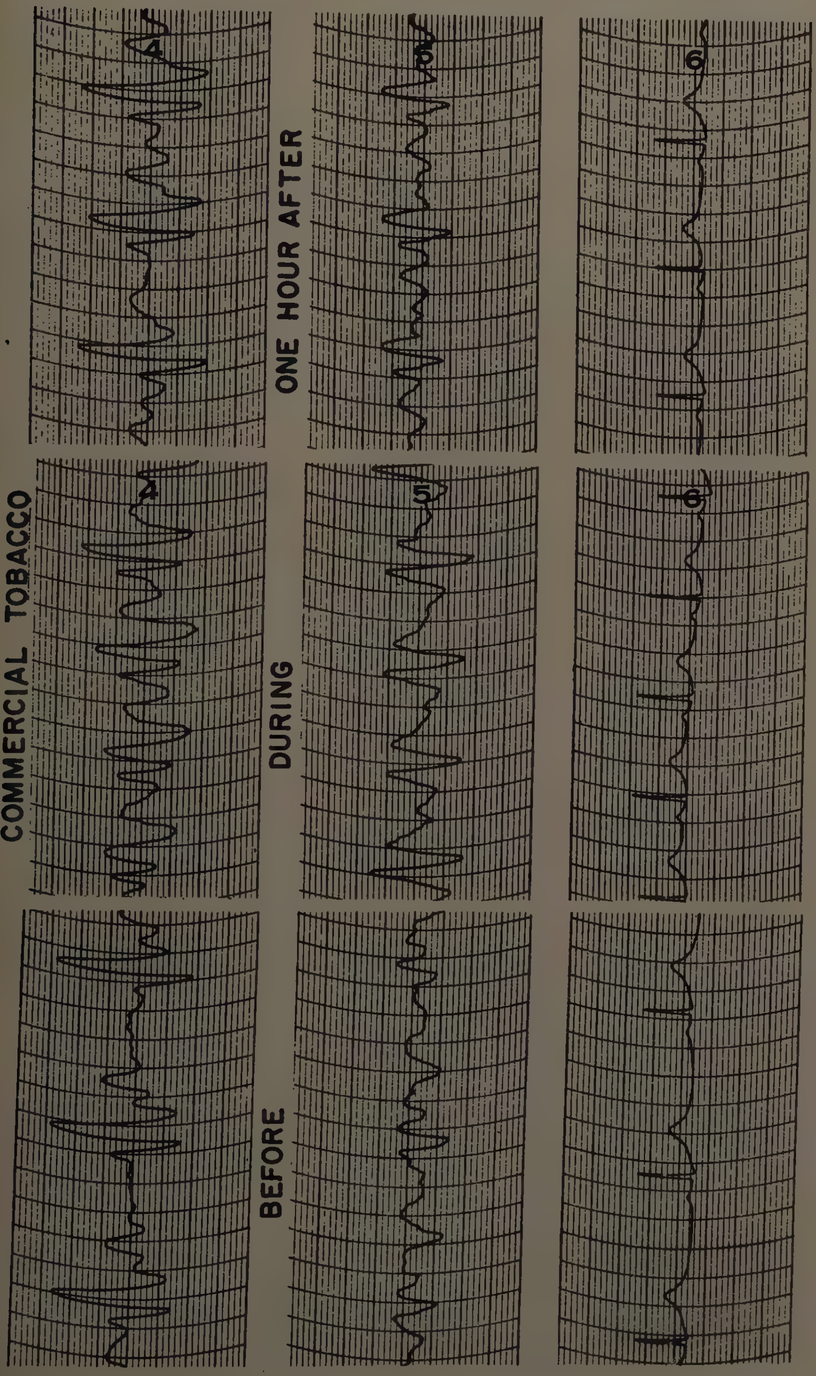

FIGURE 1. Ballistocardiogram: longitudinal component (*upper row*), horizontal component (*middle row*), and lead 2 electrocardiogram. Normal complex deteriorates 15 min. after patient begins chewing commercial tobacco. The pattern returns to normal one hour later.

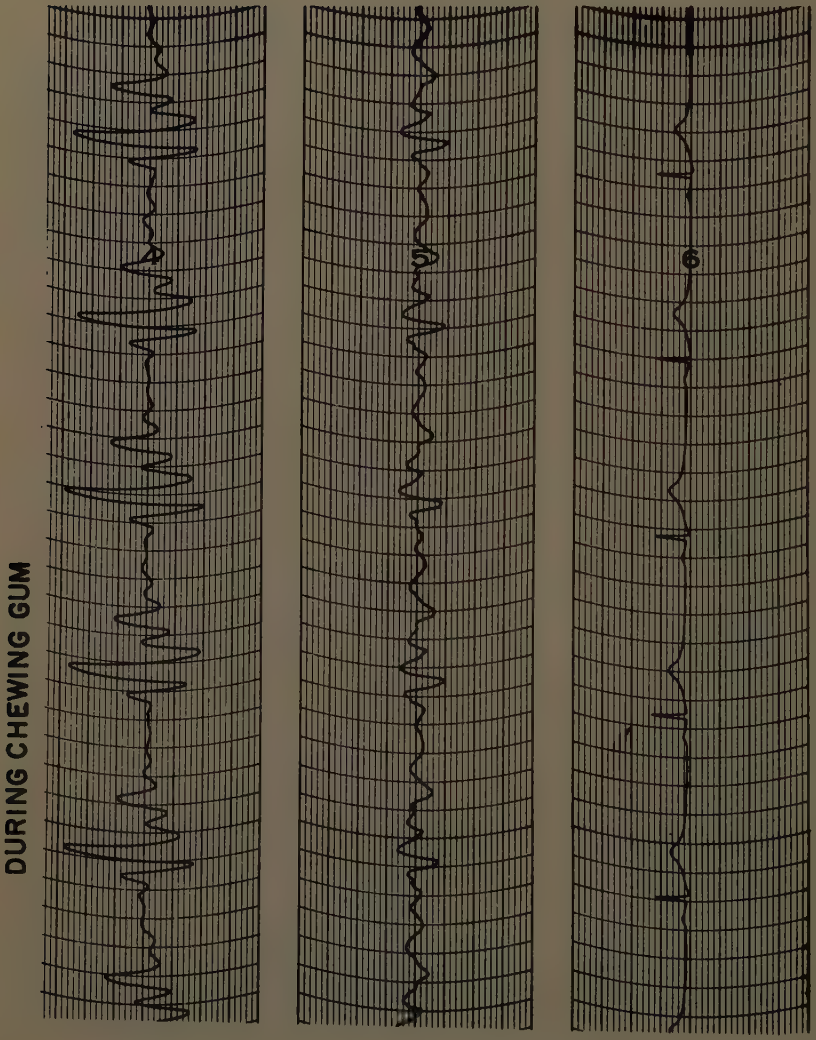

FIGURE 2. Ballistocardiograms and electrocardiograms arranged as in FIGURE 1, showing in the same patient no change after the chewing of gum for 15 min.

(85.5° F.). Eight subjects who chewed commercial tobacco showed no significant change in toe temperature, three subjects showed small increases (1.3° to 1.7° C.), and 11 showed decreases ranging from 1.1° C. to 5.3° C. With low-nicotine tobacco, 9 subjects showed no change, 4 showed slight increases (1.1° to 2.4° C.), and 7 showed decreases ranging from 1.2° to 3.6° C. Toe temperatures of 3 subjects who chewed gum did not change. The initial finger temperatures ranged from 25.3° to 38.4° C. (77.5° to 101.1° F.), with a mean of 32.5° C. (90.5° F.). The finger temperatures of six subjects who chewed commercial tobacco remained constant; there were no increases, and 17 subjects showed decreases ranging from 1.3° to 6.7° C. With low-nicotine tobacco, finger temperature increased slightly in one subject, remained constant in 6,

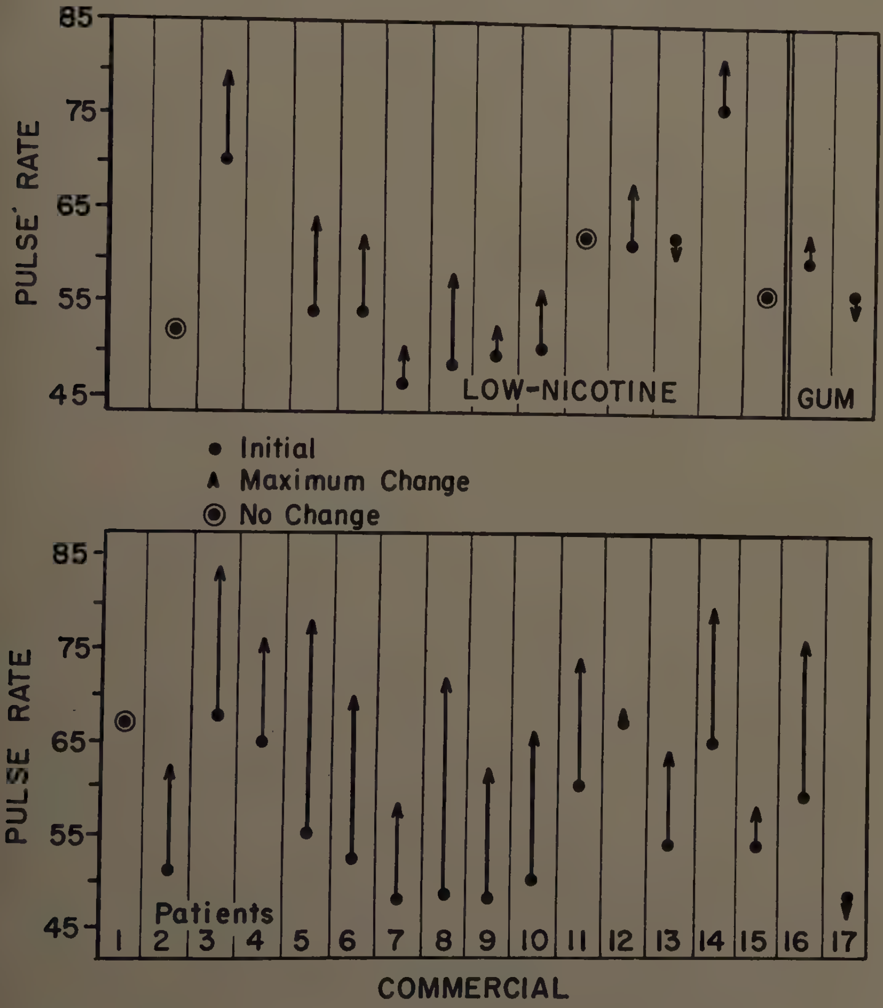

Figure 3. Effect of chewing tobacco and chewing gum on pulse rate.

and decreased in 13, the decrease ranging from 1.1° to 6.8° C. Changes were minimal in those subjects who chewed gum (FIGURES 6, 7).

CIGAR AND PIPE SMOKERS

Ballistocardiograms

Cigars. Studies were made of 12 subjects who smoked regular commercial cigars. In 9 of the 12 subjects, ballistocardiograms were normal before smoking. Three of these remained normal throughout the smoking period and after smoking. Three showed increased respiratory variation. Three, however, showed greater changes from normal, either in the form of early M or late downstroke patterns (FIGURE 8).

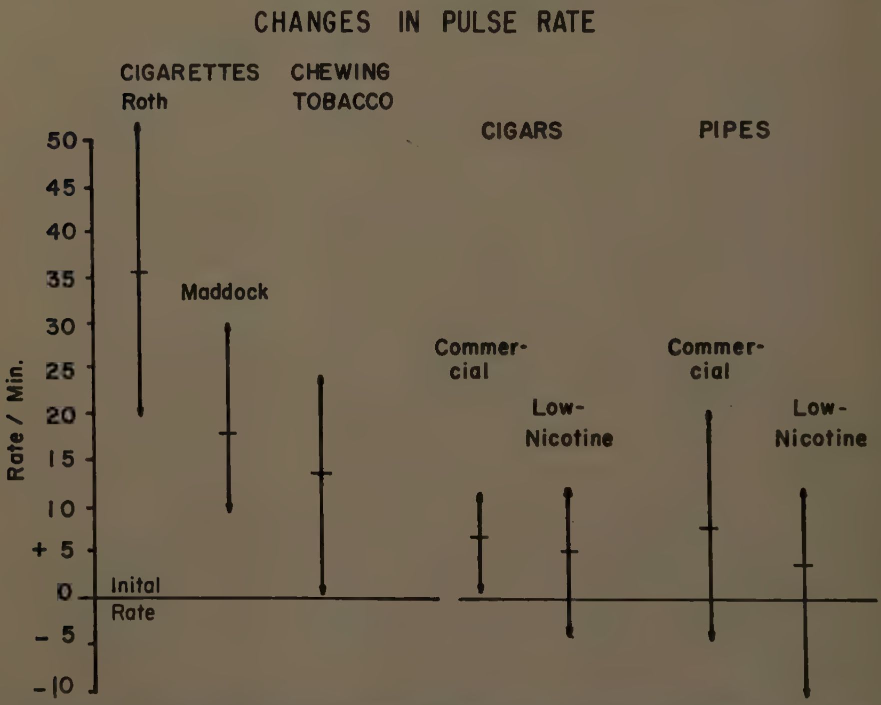

FIGURE 4. Range of pulse rate changes indicated by vertical arrowed lines. Cross bars indicate average change. Data of other workers are included for comparison.

The records of the other 3 subjects were abnormal before smoking. In 2 of these, the abnormality increased during and after smoking, and in one it lessened. The maximum changes occurred within 10 to 20 min. of the start of smoking and persisted for 20 to 30 min. or longer.

Pipes. Ten subjects were studied who smoked regular commercial tobacco. The ballistocardiograms of eight were normal before smoking. Three of these remained normal throughout; three showed only minor changes, consisting either of minor respiratory variation or changes in the amplitude of the com-

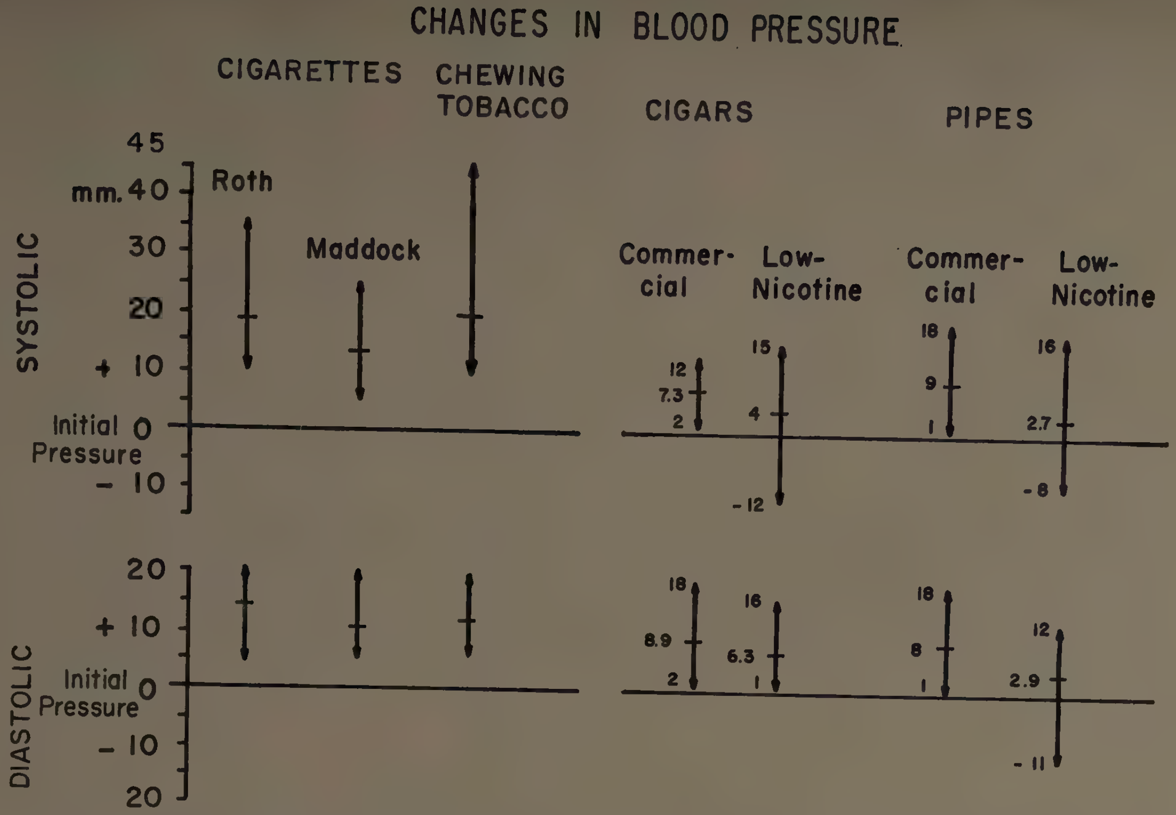

FIGURE 5. Range of blood pressure changes indicated by vertical arrowed lines. Cross bars indicate average change. Data of other workers are included for comparison.

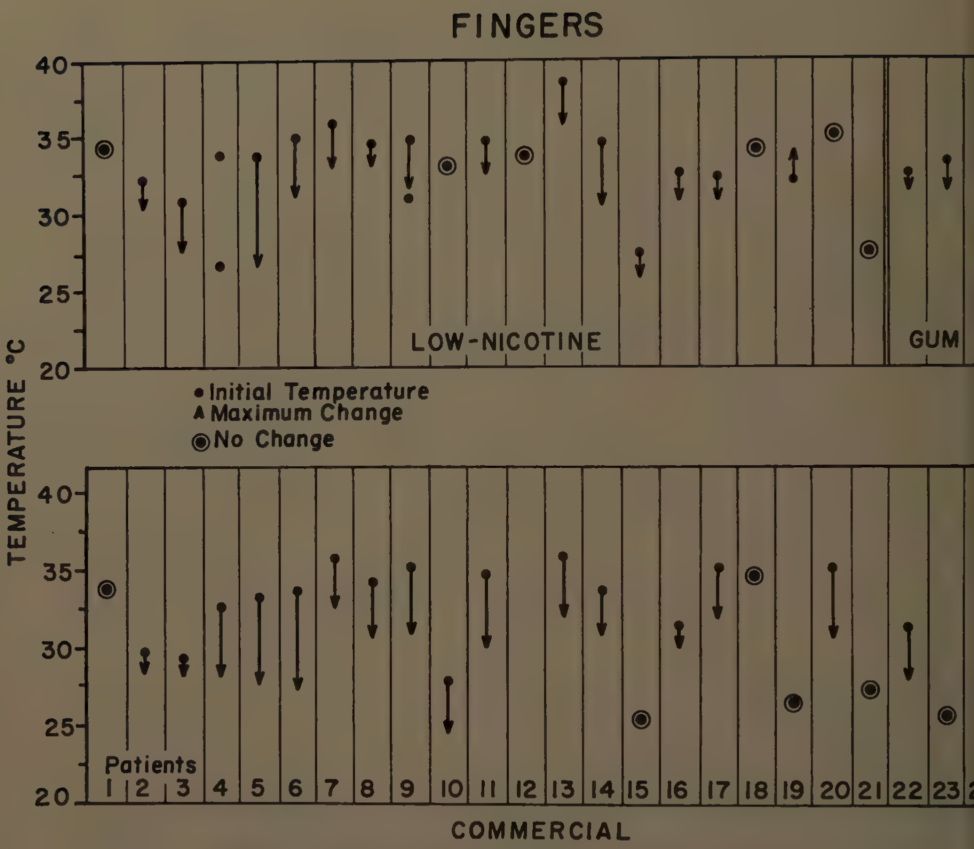

FIGURE 6. Changes in skin temperatures of fingers in subjects who chewed low-nicotine tobacco and gum (*above*) and commercial tobacco (*below*)

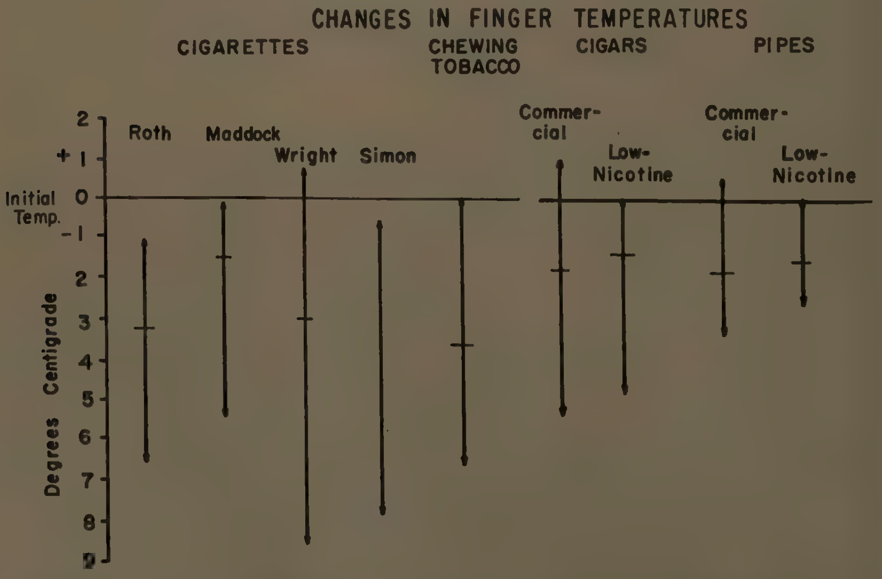

FIGURE 7. Range of skin temperature changes indicated by vertical arrowed lines. Cross bars indicate average change. Data of other workers are included for comparison.

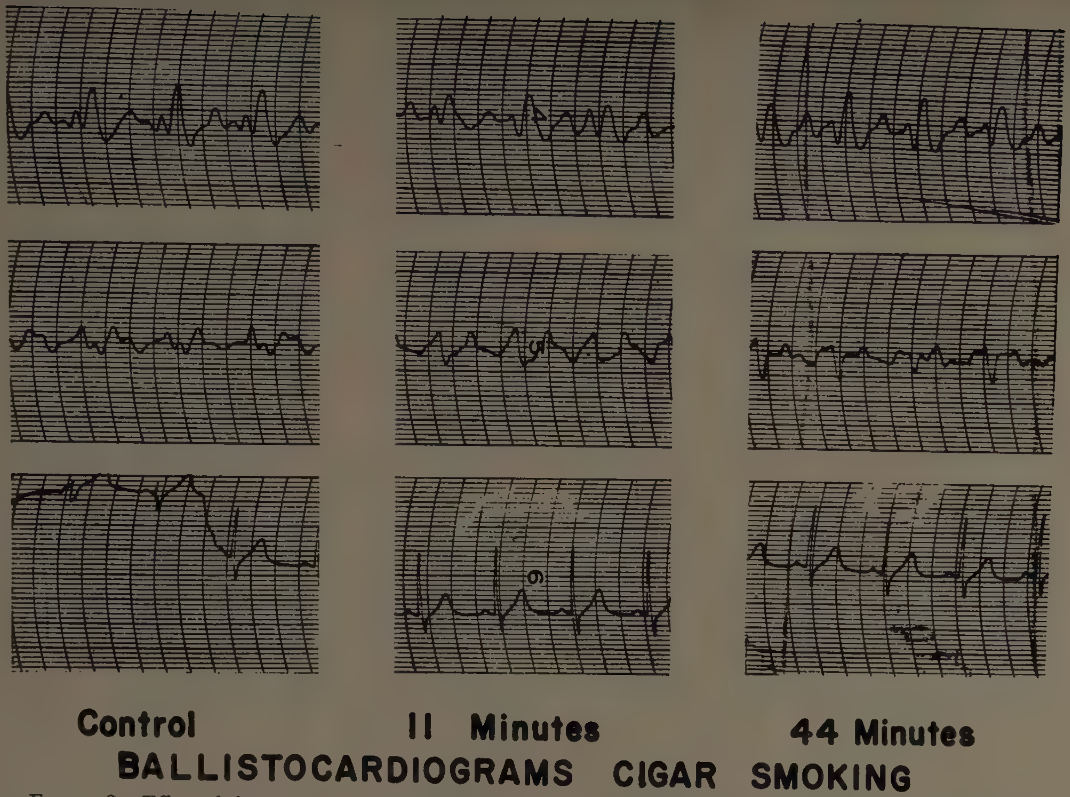

Figure 8. Effect of cigar smoking on ballistocardiogram. Top row, longitudinal component; middle row, side to side component; and bottom row, lead 1 electrocardiogram. Normal complexes (control) changing to early M pattern (*row 1*) 11 min. after start of smoking, and normal pattern reappearing 24 min. after end of smoking.

plexes. Two showed rather marked abnormality after smoking, with development of a definite early M pattern (FIGURE 9).

In 2 subjects who smoked pipes the records were abnormal before smoking. In one of these, the record showed only minimal changes during smoking, but in the other the abnormalities became much more marked. The changes occurred in from 10 to 20 min. and persisted for 20 or 30 min. or longer.

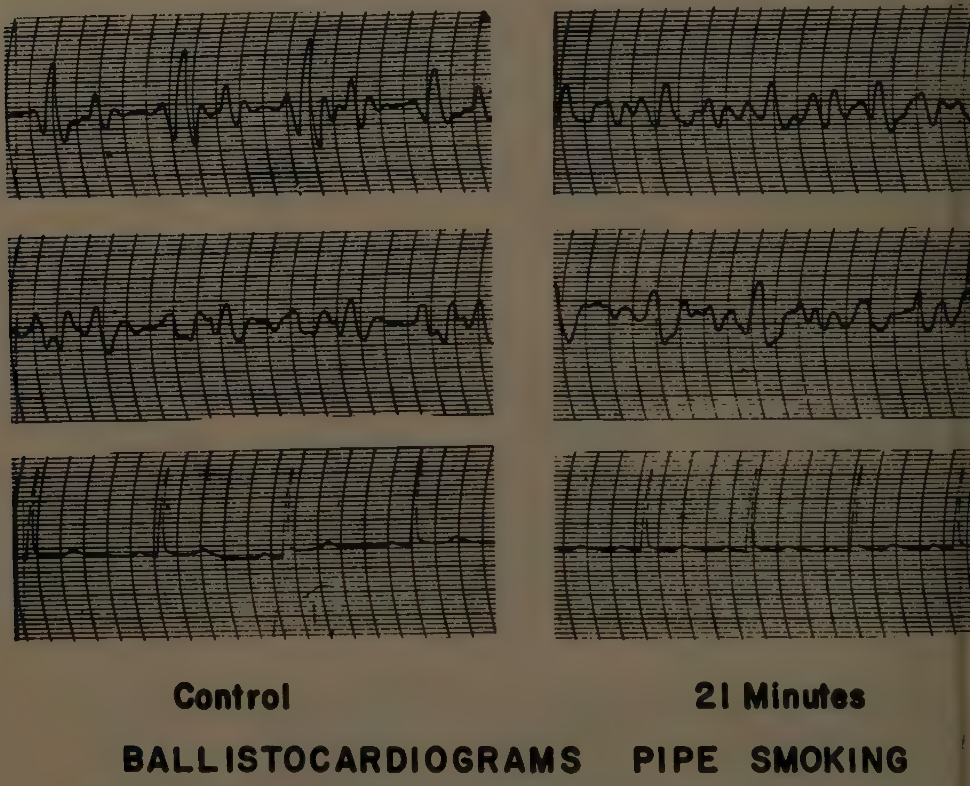

FIGURE 9. Effect of pipe smoking on ballistocardiogram. Top row, longitudinal component; middle row, side to side component; and bottom row, lead 1 electrocardiogram. Normal complexes noted during control period becoming abnormal, showing an early M pattern at end of smoking period.

Pulse Rate

Cigars. The pulse rates were counted in 15 subjects who smoked both high- and low-nicotine content cigars. In all of the subjects smoking regular commercial cigars, the pulse rate increased; the range of increase was 1 to 12 beats/min., with an average increase of 6.4 beats/min. During the smoking of the low-nicotine cigars, the pulse rate increased in 14 subjects, in the range of from 1 to 15 beats/min. In 1 subject the pulse rate decreased slightly. The average increase in these subjects was 5.3 beats/min. (FIGURE 4).

Pipes. Ten subjects smoked regular and low-nicotine tobacco in pipes.

During the smoking of the regular commercial tobacco, 9 subjects showed an increase in pulse rate, ranging from 3 to 20 beats/min.; in one subject the rate decreased 4 beats/min. The average increase was 7.4 beats/min. While smoking low-nicotine tobacco the pulse rate increased in 7 subjects with a range of 2 to 12 beats/min. In 2 subjects the heart rate decreased 4 and 10 beats/min., respectively, and in 1 the rate remained unchanged. The average increase was 4.2 beats/min. (FIGURE 4). Maximal changes in the pulse rate occurred from 2 to 30 min. after the start of smoking.

Blood Pressures

Cigars. Fifteen subjects smoked regular and low-nicotine cigars. All subjects showed an increase in both systolic and diastolic blood pressure while smoking regular cigars. The systolic blood pressure increased an average of 7.3 mm. Hg, range 2 to 12 mm., and the diastolic blood pressure increased an average of 8.9 mm. Hg, range 2 to 18 mm. (FIGURE 5).

During the smoking of low-nicotine cigars, systolic blood pressure increased in 11 subjects, range 1 to 15 mm. Hg. In 3 there was no change in systolic blood pressure, and in one the systolic blood pressure decreased. The average increase was 4 mm. Hg. The diastolic blood pressure increased in all subjects with an average increase of 6.3 mm. Hg, range 1 to 16 mm. (FIGURE 5).

Pipes. Blood pressures were measured in ten subjects smoking regular commercial and low-nicotine tobacco in pipes. During the smoking of high-nicotine tobacco, the systolic and diastolic blood pressure increased in all subjects. The average increase was 9 mm. systolic and 8 mm. diastolic with a range of 1 to 18 mm. Hg in both. During the smoking of low-nicotine tobacco in pipes, systolic blood pressure increased in 5 subjects, decreased in 2 and remained unchanged in 3. Diastolic blood pressure increased in 7 of these subjects and decreased in 3 (FIGURE 5).

Skin Temperatures

Cigars. Skin temperatures were measured in the fingers and toes of 15 subjects who smoked regular commercial cigars. The average initial temperature of the fingers was 32.3° C. Skin temperatures fell in 12 of the subjects, remained unchanged in 1, and increased slightly in 2. Eleven subjects were studied with low-nicotine cigars. The initial fingertip temperatures averaged 32.5° C. In 9 subjects the skin temperatures decreased, and in 2 subjects changes were equivocal (FIGURE 7). In both groups of observations the toe temperatures did not show any consistent pattern of change. Forehead temperatures did not change.

Pipes. Skin temperature observations were made on 10 subjects while they smoked regular commercial tobacco in pipes. The average initial temperature of the fingers was 33.2° C. Finger temperatures decreased in nine of the subjects and in one subject changes were equivocal (FIGURE 7). Nine subjects were studied with low-nicotine tobacco. The average initial finger temperature was 33.9° C. Finger temperatures decreased in eight of the subjects, and in one subject changes were equivocal. No consistent pattern of changes was noted in the feet.

Forehead temperatures did not change. Maximal changes in skin temperature occurred from 9 to 50 min. after the start of smoking.

The averages of changes with cigar and pipe smoking were almost identical. Greater decrease in finger temperature occurred in a few cigar smokers (FIGURE 7).

Sham smoking. Eight subjects who puffed unlit cigars or pipes showed no change in pulse rate, blood pressure, or ballistocardiograms. In most of these subjects changes did occur with actual smoking.

Discussion

All observers have found ballistocardiographic changes in only a small proportion of healthy young males during cigarette smoking.[1,9,10,11] However, we found changes of the ballistocardiogram in 23 of 24 subjects who chewed tobacco. Abnormalities of the ballistocardiogram occurred or increased in 8 of 12 subjects after smoking cigars and in 6 of 10 subjects after smoking pipes.

In contrast to the subjects in previous studies of cigarette smokers, none of the pipe or cigar smokers inhaled. Previous studies are not in agreement as to the percentage of nicotine absorbed by smokers who did not inhale. The most frequently cited work, that of Baumberger[12] in 1923, indicates a high percentage of nicotine absorption, even without inhaling (66–77 per cent). A more recent study by Greenberg[13] and others indicated that, without inhaling, a smaller percentage of nicotine content of smoke is retained in the body. They found 4 to 45 per cent of the nicotine was retained in smokers who did not inhale, whereas 96–98 per cent was absorbed in subjects who inhaled. This raises the question of the effect of the respiratory activity alone during smoking on the circulation.

Mulinos and Shulman[14] found that deep breathing could produce transient vasoconstriction in the hands. However none of our pipe or cigar smokers inhaled or breathed deeply. Many previous observers, including Roth,[6] Maddock and Coller,[15] Wright and Moffatt,[16] and Weatherby[17] have shown that sham smoking produced negligible changes in skin temperatures, pulse rates, and blood pressures. More recent studies by Freund[18] were confirmatory. Furthermore, Weatherby[17] found quite inconsistent, small, and variable changes in actual respiration during the act of smoking. In our own studies of sham smoking or chewing, no circulatory changes were found, even in subjects who showed changes while smoking or chewing tobacco.

Thus the data on cigar and pipe smokers indicate that failure to inhale does not prevent appearance of the usual circulatory effects of nicotine. Very few previous studies of pipe and cigar smoking were found. Maddock and Coller,[15] in a few subjects, found that, following pipe smoking, pulse, blood pressure, and skin temperature changes were similar to cigarette smokers. According to Scott,[19] nicotine levels in the blood were lower after cigar or pipe smoking than after cigarette smoking.

It should be pointed out that the amount of nicotine available for absorption varies with such factors as moisture content, combustion temperature, length of filtration, loss through expectoration, as well as the obvious factors of nicotine content of tobacco smoked or chewed and rate of smoking or chewing.

Summary and Conclusions

Cigarette smoking. In the normal young men no significant change in cardiac output was found after smoking ordinary cigarettes.

While showing no change in cardiac output, these same normal young men showed concurrent statistically-significant increases in pulse rates and decreases in skin temperatures of fingers.

In our subjects there were no changes in the form of the ballistocardiogram following cigarette smoking.

Tobacco chewing. The chewing of tobacco in a group of older men who chewed tobacco habitually produced an increase in pulse rate and blood pressure, a decrease in skin temperature of the fingers, and changes of the ballistocardiogram in almost all subjects.

Cigar and pipe smoking. The usual effects of tobacco smoking on the circulation were found (that is, an increase in pulse and blood pressure, drop in skin temperature of fingers), but were not as marked as the effects usually found following cigarette smoking or chewing tobacco.

The incidence of changes in the ballistocardiogram in this study was less than that found following chewing tobacco in a similar age group, but greater than that found following cigarette smoking in a younger age group.

The circulatory effects of cigar and pipe smoking were approximately the same.

Circulatory changes were slightly less following low-nicotine cigars and pipes compared with standard cigars and pipes.

Sham smoking or sham chewing did not produce circulatory changes.

References

1. Simon, D. L., A. Iglauer & J. R. Braunstein. 1954. The immediate effect of cigarettes on the circulation of healthy and habitual male smokers. Am. Heart J. **48:** 185–188.
2. Simon, D. L., A. Iglauer, J. R. Braunstein & R. E. Rakel. 1957. Immediate effect of chewing tobacco on circulation of habitual chewers. J. Am. Med. Assoc. **163:** 354–356.
3. Simon, D. L. & A. Iglauer. 1960. Circulatory Effects of Pipe and Cigar Smoking. Read at Regional Meeting. American College of Physicians. January 29. Columbus, Ohio.
4. Braunstein, J. R., C. E. Oelker & R. C. Gowdy. 1950. Design of two dimensional ballistocardiograph. J. Clin. Invest. **29:** 1219–1226.
5. Grollman, A. 1932. The Cardiac Output of Man in Health and Disease. Thomas. Springfield, Ill.
6. Roth, G. M., J. B. McDonald & C. Sheard. 1944. The effect of smoking cigarets and of intravenous administration of nicotine on electrocardiogram, basal metabolic rate, cutaneous temperature, blood pressure and pulse rate of normal persons. J. Am. Med. Assoc. **125:** 761–767.
7. Engelhardt, A. & G. Stüttgen. 1955. Zur Analyse Hauttemperatursenkender Wirkung des Tabakrauches. Arzneimittel-Forsch. **5:** 93–97.
8. Maddock, W. G., R. L. Malcolm & F. A. Coller. 1936. Thromboangiitis obliterans and tobacco. The influence of sex, race, and skin sensitivity to tobacco on cardiovascular responses to smoking. Am. Heart J. **12:** 46.
9. Dock, W., H. Mandelbaum & R. A. Mandelbaum. 1953. Ballistocardiography. Mosby. St. Louis, Mo.
10. Levy, R. L., M. N. Boyle, R. Wegria, R. T. Cathcart & J. L. Nickerson. 1946. Some effects of the intravenous injection of nicotine on the circulation in normal persons and in patients with cardiovascular disease. Trans. Assoc. Am. Physicians. **59:** 177.
11. Henderson, C. B. 1953. Ballistocardiograms after cigarette smoking in health and in coronary heart disease. Brit. Heart J. **15:** 278.

12*a*. BAUMBERGER, J. P. 1923. The carbon monoxide content of tobacco smoke and its absorption on inhalation. J. Pharmacol. Exptl. Therap. **21:** 23.

12*b*. BAUMBERGER, J. P. 1923. The nicotine content of tobacco smoke. *Ibid.* : 35.

12*c*. BAUMBERGER, J. P. 1923. The amount of smoke produced from tobacco and its absorption in smoking as determined by electrical precipitation. *Ibid.* : 47.

13. GREENBERG, L. A., D. LESTER & H. W. HAGGARD. 1952. The absorption of nicotine in tobacco smoking. J. Pharmacol. Exptl. Therap. **104:** 162–167.

14. MULINOS, M. C. & I. SHULMAN. 1940. The effects of cigarette smoking and deep breathing on the peripheral vascular system. Am. J. Med. Sci. **199:** 708.

15. MADDOCK, W. G. & F. A. COLLER. 1933. Peripheral vasoconstriction by tobacco and its relation to thrombo-angiitis obliterans. Ann. Surg. **98:** 70.

16. WRIGHT, I. S. & D. MOFFATT. 1934. The effects of tobacco on the peripheral vascular system. Further studies. J. Am. Med. Assoc. **103:** 318.

17. WEATHERBY, J. H. 1942. Skin temperature changes caused by smoking and other sympathomimetic stimuli. Am. Heart J. **24:** 17.

18. FREUND, J. 1957. Effects of cigarette smoking on the normal male peripheral circulation, utilizing multitechnical procedures. Circulation. **16:** 883.

19. SCOTT, R. B. 1952. Some medical aspects of tobacco-smoking. Brit. Med. J. **1:** 671.

RESPONSES OF THE PERIPHERAL VEINS IN MAN TO THE INTRAVENOUS ADMINISTRATION OF NICOTINE*

John W. Eckstein and A. W. Horsley
Department of Internal Medicine, State University of Iowa College of Medicine, Iowa City, Iowa

During previous plethysmographic studies[1] it was noted that smoking led to reductions in the resting volume of the extremity. This observation suggested that smoking causes venous constriction, since the veins and venules contain most of the blood. The present studies were undertaken to determine if the intravenous administration of nicotine causes an increase in venomotor tone.

Methods and Procedure

The first experiments were performed on 12 healthy young men. All were habitual smokers who used 20 to 60 cigarettes per day. They did not smoke during the two-hour period before reporting to the laboratory. Tests were done with subjects lying supine. Room temperature was 80° F. Venous tone was measured with a plethysmograph on the left forearm. Venous pressure was measured in the antecubital vein of the dependent right arm in 5 of the 12 experiments. Arterial blood pressure was determined by the cuff method and heart rate was obtained by counting the radial pulse. After control values were established 1.0 mg. of nicotine base in 5.0 ml. of normal saline was injected through an indwelling needle into an ankle vein. The observations were then continued until control values returned. In 7 experiments the nicotine injection was repeated 5 to 7 min. after intravenous administration of 5.0 mg. of phentolamine methanesulfonate (Regitine).

Venous tone was measured by a modification of the method described previously by Wood and Eckstein.[2] The forearm is enclosed in a tall plethysmograph and water is added, so that the pressure it exerts on the arm is more than venous pressure but less than arterial pressure. Arterial inflow drives venous pressure in the forearm to a height greater than that of the water. The difference between the pressure within the veins and the pressure surrounding them is the transmural pressure. Under these conditions it is a low value (0.5 to 1.0 mm. Hg) and constant in a given subject.[2] The volume of blood in the veins at this low transmural pressure also is small and constant.[3] The origin of the venous pressure-volume curves is the venous volume which exists at this low level of transmural pressure. Since the volume and pressure values are so small, for practical purposes they are regarded as zero.

To observe the pressure-volume relationships, transmural pressure in the forearm veins is increased from "zero" to 30 mm. Hg in increments of 5.0 mm. Hg by inflating a pneumatic cuff on the arm proximal to the plethysmograph. The increase in forearm venous volume caused by each increment of pressure is measured and recorded. Curves are constructed by plotting venous volume, in ml./100 ml. of forearm tissue, against transmural pressure (FIGURE 1). The

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curves are convex toward the volume axis. If the veins become less distensible the curve falls nearer the pressure axis; if they become more distensible the curve falls nearer the volume axis. The final point on the curve, the volume at a transmural pressure of 30 mm. Hg, is termed arbitrarily the venous distensibility. A high value is associated with reduced tone, and a low value with increased tone. Since the venous pressure-volume characteristics are the same in both arms, the naturally occurring venous volume of the forearm is the volume coordinate of the point on the curve that corresponds to the venous pressure (equals transmural pressure) measured in the arm not in the plethysmograph. The natural volume is determined by drawing a perpendicular from the measured value on the pressure axis to the point of intersection with the curve (FIGURE 1).

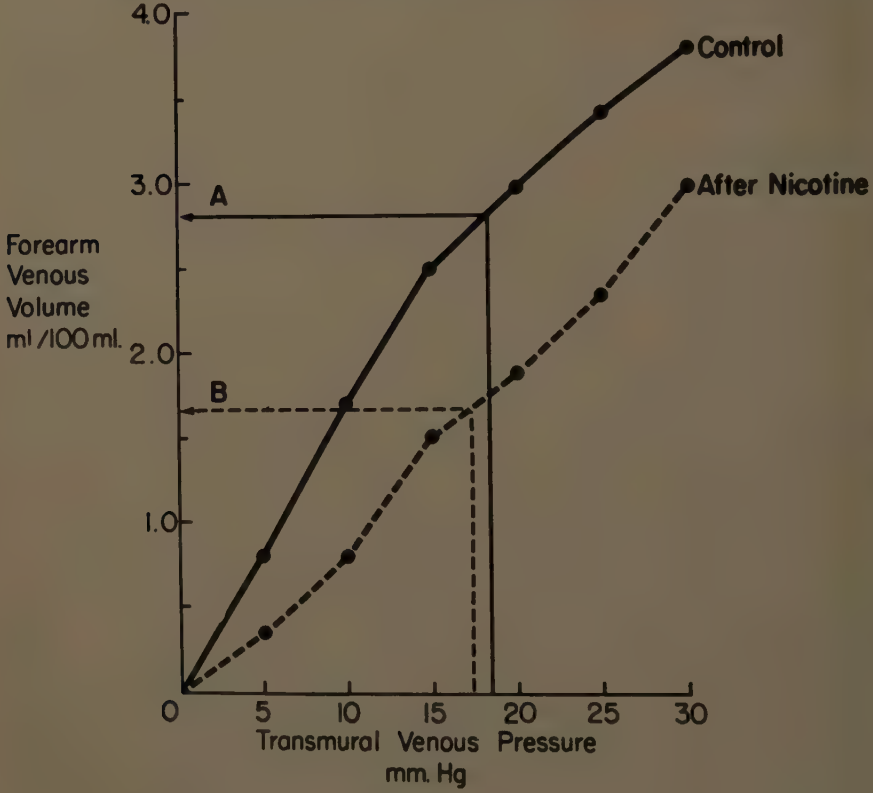

FIGURE 1.

Forearm volume changes were measured by means of two partially immersed electrodes[4] * that recorded changes in the height of the water in a vertical cylinder attached to the top of the plethysmograph. The diameter of the cylinder was such that maximal changes in limb volume raised the water level

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* Constructed by the Sanborn Company, Waltham, Mass.

less than 5 mm. This increase in water level was not sufficient to cause a measurable error in transmural pressure in the veins. A long plethysmograph rather than the previously described tandem instrument[2] was used. With a long box, the incompletely pressurized zone of tissue at the proximal end contributes only a negligible fraction to the total volume change within the plethysmograph unless the limb diameter is unusually large.

TABLE 1

CHANGES IN FOREARM VENOUS DISTENSIBILITY AFTER INTRAVENOUS INJECTION OF NICOTINE* BEFORE AND AFTER ADMINISTRATION OF PHENTOLAMINE**

Experiment number	Venous distensibility (ml./100 ml.)			Mean blood pressure (mm. Hg)			Pulse rate (beats/min.)		
	C†	N††	P-N‡	C	N	P-N	C	N	P-N
1	4.0	3.2	3.4	80	108	94	62	80	92
2	3.8	3.0	3.6	96	105	108	76	96	96
3	3.2	2.6	2.5	80	98	105	95	104	100
4	4.5	3.4	3.5	88	100	96	60	92	92
5	3.9	2.8	2.6	88	100	97	80	84	84
6	3.5	3.5	3.4	83	88	93	72	72	96
7	4.9	4.2	4.8	86	95	90	64	100	104
8	3.8	3.4		91	105		72	100	
9	3.6	3.0		96	100		60	84	
10	4.1	3.5		85	101		64	72	
11	4.0	3.6		103	111		84	105	
12	4.7	4.3		108	117		88	96	
Experiments 1–12									
Mean	4.0	3.4		90	102		73	90	
Standard error§	0.09			1.9			3.3		
Probability	<0.001			<0.001			<0.001		
Experiments 1–7									
Mean		3.2	3.4		99	98		90	95
Standard error§		0.13			2.7			3.7	
Probability		<0.2			<0.5			<0.3	

* Nicotine dose, 1 mg. of base.

** Phentolamine methanesulfonate dose, 5.0 mg. of salt.

† Value obtained during control period.

†† Value obtained after nicotine injection.

‡ Value obtained after nicotine injection following phentolamine administration.

§ Standard error of the mean difference.

In the second group of experiments nicotine in normal saline was infused into an ankle vein at constant rates for periods of 10 min. Toe temperature, radial pulse, venous pressure, and respirations were recorded continuously. Arterial pressure and venous tone were measured intermittently, and subjects were asked about unusual symptoms after the infusion was discontinued.

Results

Symptoms occurred in each of the 12 subjects when 1.0 mg. of nicotine was injected intravenously. The most frequent symptom was lightheadedness or dizziness. Two subjects found this sensation to be pleasant. None of them complained of nausea. Seven complained of transient discomfort in the extremity with the injection. Three complained of severe aching pain that involved the entire lower extremity with a "stocking-type" distribution. The remainder described mild to moderate aching below the knee. This appeared to last about 1 min. Irregular breathing and a transient increase in respiratory rate were noted in almost all subjects. Pallor was noted in about one half of the subjects, and several of them were observed to have pilomotor changes and sweating.

TABLE 2

CHANGES IN FOREARM VENOUS VOLUME AFTER INTRAVENOUS INJECTION OF NICOTINE*

Experiment number	Venous pressure (mm. Hg)		Venous distensibility (ml./100 ml.)		Venous volume (ml./100 ml.)	
	C†	N‡	C	N	C	N
8	16.0	17.5	3.8	2.4	3.0	1.5
9	18.6	18.6	3.6	3.0	3.0	1.8
10	13.5	12.5	4.1	3.5	2.7	2.3
11	12.3	12.3	4.0	3.6	2.7	1.9
12	11.3	12.0	4.7	4.3	2.6	2.7
Mean	14.3	14.5	4.0	3.4	2.8	2.0
Standard error§	0.42		0.18		0.28	
Probability	<0.7		<0.02		<0.05	

* Nicotine dose, 1 mg. of base.
† Values during control period.
‡ Values after nicotine injection.
§ Standard error of the mean difference.

TABLE 3

FREQUENCY OF CHANGE IN CERTAIN CARDIORESPIRATORY FUNCTIONS WITH TEN-MINUTE INFUSIONS OF NICOTINE

Number of subjects	Total nicotine dose	Toe temperature	Heart rate	Arterial pressure	Venous pressure	Venous distensibility	Respiratory rate	Symptoms
	Mg.	Number decreased	Number increased	Number increased	Number increased	Number decreased	Number increased	Number reported
8	0.5 to 1.0	0	0	0	0	0	1	0
8	1.1 to 2.0	3	3	1	0	1	1	0
6	2.4 to 3.1	3	2	2	2	1	1	2
6	3.4 to 4.4	6	6	4	1	6	3	6

Mean arterial pressure (diastolic plus one third of the pulse pressure) increased an average of 12 mm. Hg (TABLE 1), and pulse rate increased an average of 17 beats/min. The recorded values for pressure and pulse rate are those observed at the completion of the venous pressure-volume curves. These curves were completed 3 to 4 min. after the injection. The greatest blood pressure and heart rate values were noted within a few seconds after the injection and were somewhat higher than the reported values. An increase in venomotor tone occurred in 11 of the 12 experiments. The venous volume at 30 mm. Hg averaged 4.0 ml./100 ml. of tissue during control periods and 3.4 ml. after nicotine. The responses to the drug were not altered appreciably by prior administration of phentolamine. Venous pressure was measured in 5 of the 12 experiments (TABLE 2) and no consistent change was noted. When forearm venous volume was estimated by drawing a perpendicular from the appropriate point on the pressure axis (FIGURE 1) it was found that the increased tone was sufficient to "push" blood from the forearm. The maximal venous response occurred promptly after the drug was given, and the effect disappeared completely in about 20 min.

In the second group of experiments nicotine was infused at a constant rate for 10 min. The total dose varied from 0.5 to 4.4 mg. Twenty-four infusions were given to 9 subjects in an effort to determine the approximate dose necessary to produce cardiorespiratory changes (TABLE 3). Doses less than 3.0 mg. in 10 min. produced few symptoms, with only occasional decreases in toe temperature and increases in heart rate. Doses of 3.4 to 4.4 mg. in 10 min. regularly produced symptoms and changes in toe temperature, heart rate, and venous tone. Arterial pressure increased only slightly and respiratory rate increased in 3 of the 6 experiments (TABLE 2). Several subjects complained of leg discomfort during the infusion. This was not severe and disappeared promptly when the infusion was discontinued. In 3 subjects not included in this group it was necessary to discontinue the infusion because of pain before 5 min. had elapsed. The maximal concentration of nicotine in the infusate was approximately 0.03 mg./ml. Two of these last 3 subjects developed moderately severe superficial thrombophlebitis at the site of infusion.

Summary

In these experiments venous tone increased regularly after the intravenous injection of 1.0 mg. nicotine. It also occurred regularly when doses in excess of 3.4 mg. were infused in 10 min. The mechanism by which this venous response is produced is not clear. Phentolamine in a dose sufficient to modify the venous response to epinephrine infusion of 15 μg./min.[5] did not alter the response to nicotine. This suggests that circulating epinephrine is not an important factor. A response from a central effect of the nicotine is certainly possible, for an increase in venomotor tone was rarely observed in the absence of pain or some other unpleasant symptom. The sweating, pallor, and tachycardia that accompanied the venous response, however, suggest that it may be caused by the ganglionic stimulating properties of nicotine.

References

1. ECKSTEIN, J. W., J. E. WOOD & R. W. WILKINS. 1957. Comparative vasoconstrictor effects of smoking cigarettes in warm and cool environments and before and after abstinence from tobacco. Am. Heart J. **53:** 455.
2. WOOD, J. E. & J. W. ECKSTEIN. 1958. A tandem forearm plethysmograph for study of acute responses of the peripheral veins of man. The effect of environmental and local temperature change, and the effect of pooling blood in the extremities. J. Clin. Invest. **37:** 41.
3 LITTER, J. & J. E. WOOD. 1954. The volume and distribution of blood in the human leg measured *in vivo.* I. The effects of graded external pressure. J. Clin. Invest. **33:** 798.
4. COOPER, K. E. & D. MCK. KERSLAKE. 1951. An electrical volume recorder for use with plethysmographs. J. Physiol. London. **114:** 1.
5. ECKSTEIN, J. W. & W. K. HAMILTON. 1957. The pressure-volume responses of human forearm veins during epinephrine and norepinephrine infusion. J. Clin. Invest. **36:** 1663.

TOBACCO HYPERSENSITIVITY

Vincent J. Fontana
Department of Pediatrics, New York University Medical Center, College of Medicine, New York, N. Y.

In the past thirty years, particularly in the last several years, the possibility that tobacco or its products might be a cause of disease has been briskly discussed. The questions of whether allergy to tobacco does occur and, if it does, of how intimately it is associated with recognizable peripheral vascular symptoms, remain to be answered. The investigations of Sulzberger[1] and the reports of Harkavy *et al.*,[2] about 30 years ago, were the first to indicate that a sensitivity reaction to tobacco might be responsible for certain vascular diseases, especially thromboangiitis obliterans. On the other hand, reports by Trasoff *et al.*,[3] Chobot,[4] and Westcott and Wright[5] failed to confirm the contention that the skin reaction produced by tobacco extract was a specific response designating a sensitization process of the blood vessels.

These divergent views and findings by competent investigators in the field demanded a reappraisal of the sensitizing potential of the allergen, tobacco, on the human vascular system and ascertainment of the true incidence and significance of the positive intradermal reaction to tobacco extract. Many of the thoughts expressed in this report are based on a study of more than 1000 volunteers, including adults and children, tested with 5 different tobacco extracts. Approximately 5000 intradermal reactions to tobacco extract were observed and studied in relation to personal allergy, familial allergy, symptoms related to tobacco smoking, peripheral vascular symptoms and, finally, plethysmographic measurements of blood flow to the lower extremities and automatic reading of surface temperature.

While the observations of Sulzberger, Harkavy, and others have verified the immunologic specificity of the skin reaction to tobacco, the clinical significance of the positive skin test is not always obvious. It must be admitted that certain difficulties are encountered in using any type of skin test as a means of diagnosis. The more important factors that must be considered in evaluating a positive intradermal reaction include the preparation and reliability of the extracts, the method of testing, the importance of the reaction in a single individual, and the correlation of the positive skin reaction to the allergen with the patient's symptomatology. The tobacco extracts used in this study have been prepared by workers experienced in this field. Care was taken in the performance of the intradermal test and in the interpretation of the results in order to reduce to a minimum the possibility of nonspecific false positive reactions and other results of experimental error.

Before presenting the results of this investigation, I shall state some fundamental immunologic concepts that prompted my belief that certain tobacco effects are based on a specific allergic susceptibility of particular individuals, rather than on toxic products in the tobacco smoke.

It is known that most forms of allergy affecting different parts of the body can be verified in many instances by observations of the skin after introduction of the allergen responsible for the disease. This is the first concept of fixed specific skin hypersensitivity. The spontaneous allergies are marked by a hereditary tendency and the ability to produce skin-sensitizing antibodies that are responsible for positive tests with the suspected allergen. These skin-sensitizing antibodies relate the presence of sensitization in a variety of "shock organs."

This brings me to the second concept, the susceptibility of certain organs or parts of an organ of the body to certain allergens. I am aware of sensitivity to pollens and of their predilection for the mucous membranes of the nasopharynx and the bronchial tree, with resulting seasonal coryza or bronchial asthma. Certain circulating allergens, particularly in foods and drugs, will cause disturbances of the skin, producing rashes, urticarial lesions and, at times, severe angiodema. Each allergen seems to have a particular attraction for certain tissues.

According to the third concept, the results of antigen-antibody reactions bring about definite damage to the organ or parts of the organ affected. The Arthus phenomenon is a classical example of this concept. In recent years we have become more and more aware of organic damage, frequently permanent, that one encounters in the field of drug sensitivity. Among the most important effects in this area are those described by Rich,[6] reporting arteritis with fibrinoid changes due to drug reactions. Various blood dyscrasias have also been attributed to drug sensitivity.

These fundamental beliefs led me to interpret my findings to indicate the existence of sensitivity to tobacco in susceptible individuals, manifested by a positive skin test to the tobacco, with the allergen having a predilection for the vascular system.

The volunteers were skin-tested intracutaneously with the five different tobacco extracts. Approximately 0.02 cc. of the excitant was injected intradermally, and the site was observed for at least 20 min. to note the result of the test. The skin-test results were interpreted by the standards* proposed by Cooke.[7]

To eliminate questionable or irritant reactions, only the moderately and markedly positive reactions were considered positive for the purposes of this study.

The tobacco extracts were prepared by Margaret B. Strauss, Allergy Laboratory, University Hospital, New York, N. Y. Burley, Virginia, and Turkish cured tobaccos were procured from a reputable tobacconist who could assure their purity of type for the preparation of these individual tobaccos.

The usual routine in preparing an extract of allergen is to treat the crude mass with an organic solvent such as acetone, ether, toluene, or Sovasol in order to remove as much oil, fat, and coloring matter as possible before the aqueous extraction. In general, the oil and dye contain no antigenic fraction important to the inhalant type of allergies, and their removal produces a superior extract because it is clearer and less colored. The tobacco mass is then extracted in buffered saline (*p*H 7.0) for 2 days. After infiltration, the extract is dialyzed against buffered saline for 72 hours and then concentrated by allowing the extract, in cellophane sausage casing, to hang in front of an electric fan. After Seitz filtration and sterility tests, each extract was standardized on a protein nitrogen basis, according to the method of Stull and Cooke.[8] The tobacco extracts used for testing were in 1000 units phosphotungstic-acid-precipitable nitrogen strength.

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* Original wheal negative reaction: 0.2 to 0.3 cm. wheal. Slight reaction: 0.4 to 0.6 cm. wheal. Moderate reaction: 1 to 1.5 cm. wheal. Marked reaction: 2 plus cm., with pseudopodia.

Eighty healthy adults tested with tobacco extract were studied in the New York University Research Service at Goldwater Memorial Hospital. The vascular responses of these persons were tested by automatic recording of surface temperature and by large-limb venous occlusion plethysmograph in a room with constant temperature and humidity, under environmental conditions set at 20° and 25° C., with 55 per cent humidity. Measurements were taken before the subjects smoked a cigarette, and at 2-, 6-, and 20-min. intervals following the onset of the smoking procedure. Changes in blood flow greater than 2 cc./100 ml. of tissue per minute, and changes in surface temperature of at least 2° C., were considered significant.

The results of testing 641 healthy volunteers and 294 children with 5 different tobacco extracts, Burley, Virginia, Turkish, mixed (nondefatted), and mixed (defatted), indicate the following:

1) It was found that about 15 per cent of 641 adults reacted in a positive manner to one or more of the tobacco extracts. The percentage of reactions was essentially the same in smokers as in nonsmokers.

2) An increased incidence of peripheral vascular symptoms was noted in the smokers with positive reactions to the tobacco, as compared with the smokers who did not react on skin-testing with the tobacco.

3) Volunteers with positive reactions to the tobacco extracts reported a history of nontobacco-related allergic manifestation more frequently than the group with negative reactions.

4) Of the smokers with positive skin reactions to tobacco extracts 33 per cent gave a personal history of allergic manifestations and peripheral vascular symptoms, as compared with 10 per cent of the smokers who did not react to the skin test with tobacco extracts.

5) Eighty of the smokers with positive reactions to tobacco extracts were investigated further for circulatory disturbances by means of plethysmographic measurements of blood flow to the lower extremities and automatic recording of surface temperature. These tests were performed before, during, and after the smoking of a mixed-tobacco cigarette. These experiments indicated that, on smoking the mixed-tobacco cigarette, 23 per cent of the smokers with positive skin tests to tobacco experienced changes in peripheral circulation indicated by skin temperature, as compared to smokers with negative skin tests, of whom 4 per cent had changes in skin temperature.

6) In a group of 294 children between the ages of 1 and 5 years tested with the tobacco extracts, 11.5 per cent reacted in a positive manner to one or more of the tobacco extracts. In the small group of allergic children tested, 64 per cent gave evidence of skin sensitivity to the tobacco extracts as compared with 6 per cent of a nonallergic control group.

7) It was found that a tobacco-positive individual could be sensitive to one or more types of tobacco but not sensitive to any other types.

8) Sex and age did not seem to play any role in the incidence of reactions to tobacco in the groups tested.

I have interpreted these findings to indicate that if the positive test to tobacco were my only evidence I could not conclude that tobacco sensitization could be responsible for certain vascular diseases. However, when correlated consistently and closely with peripheral vascular symptoms, tobacco symptoms, and personal history of allergic manifestations, it is felt that skin testing with tobacco extracts serves as a useful "screening test" to aid in determining the possible importance of tobacco as an etiological factor in certain forms of vascular disease.

Acknowledgments

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References

1. Sulzberger, M. B. 1933. Studies in tobacco hypersensitivity: thromboangiitis obliterans with positive urticarial skin reactions and negative reagin findings. J. Immunol. **24:** 425.
2. Harkavy, J., S. Hebald & S. Silbert. 1932. Tobacco sensitiveness in thromboangiitis obliterans. Proc. Soc. Exptl. Biol. Med. **30:** 104.
3. Trasoff, A., G. Blumstein & M. Marks. 1936. The immunologic aspect of tobacco in thromboangiitis obliterans and coronary heart disease. J. Allergy. **7:** 250.
4. Chobot, R. 1935. The significance of tobacco reactions in allergic children. J. Allergy. **6:** 383.
5. Westcott, H. F., & I. Wright. 1938. Tobacco allergy and thromboangiitis obliterans. J. Allergy. **9:** 555.
6. Rich, A. R. 1942. The role of hypersensitivity in periarteritis nodosa as indicated by 7 cases developing during serum sickness and sulfonamide therapy. Bull. Johns Hopkins Hosp. **71:** 323.
7. Cooke, R. A. 1947. Allergy in Theory and Practice. : 494. Saunders. Philadelphia, Pa.
8. Stull, A., R. A. Cooke & J. Tennant. 1933. The allergen content of pollen extracts, its determination and its deterioration. J. Allergy. **4:** 455.

TOBACCO HYPERSENSITIVITY: PERIPHERAL CIRCULATORY IMPLICATIONS

W. Redisch, K. Meckeler, W. Brown, J. M. Steele
New York University School of Medicine, New York, N. Y.

About 30 years ago[1,2,3] it was found that about 1 of 4 persons tested showed a decrease in surface temperature in response to smoking a cigarette. Later it was reported[4,5] that plethysmographically measured blood flow was diminished in about the same percentage of test subjects after smoking. Testing of skin sensitivity to tobacco extracts yielded 20 to 30 per cent positive results.[6,7,8] The similarity in percentage figures obtained was responsible for an investigation of possible relationship between skin sensitivity to tobacco extracts and vascular responses to the smoking of tobacco.

In an initial study of 80 healthy smokers, one significant correlation was apparent: almost 90 per cent of those who had a negative skin test failed to show a decrease in total blood flow to the lower extremity.

Since, however, blood flow responses to smoking observed in the lower extremity seemed erratic in that some test subjects showed unequivocal increase in total flow, it soon became evident that it was necessary to devise at least a rough separation of skin flow and muscle flow. A method of differential plethysmography has been developed in our laboratory on the basis of the rather constant difference between skin-mass/muscle-mass ratio in the foot and in the leg. The formula permits a rough estimation of skin flow and muscle flow by correcting for the most obvious errors (FIGURE 1). To be reasonably sure that differences were significant, we considered only changes of more than 30 per cent as important.

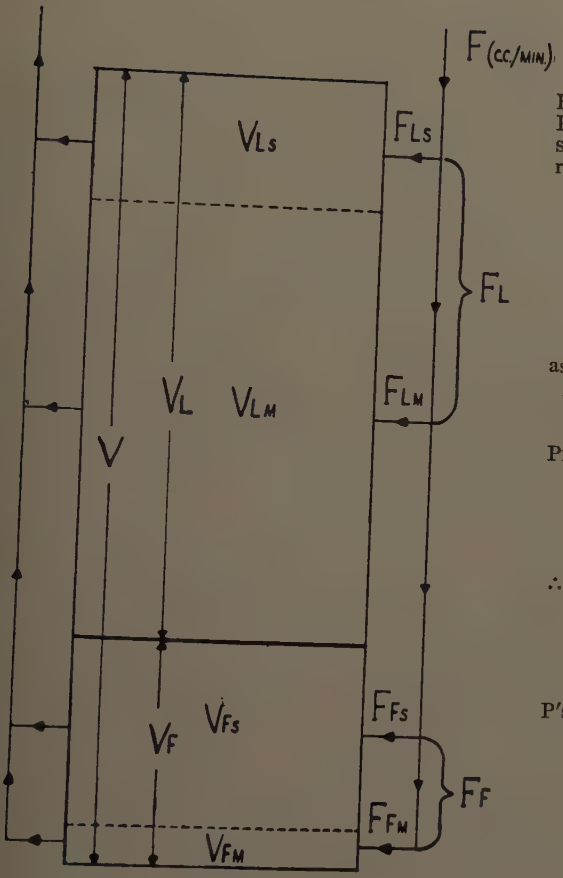
Thus far 32 subjects, comprising 23 males and 9 females, have been tested with this method. Their ages ranged from 24 to 87, the average being 55. Seven of the 32 subjects exhibited some evidence of obliterative arteriosclerosis, while the remainder had no demonstrable vascular disease.

Seven of the group were significantly sensitive to one or more specific tobacco extracts. Skin and muscle flow responses of these 7 subjects were tested for the smoking of tobacco types to which their skin was sensitive. None showed an increase in skin flow, 1 listed as no change in skin flow had, in fact, an insignificant decrease, and 6 had a significant decrease (TABLE 1).

In contrast, of the 25 subjects who showed no skin sensitivity, 8 showed an increase in skin flow, 15 showed no change and only 2 had a decrease (TABLE 2).

Surface temperatures were recorded quasi-continuously in all experiments until they became constant, to insure that adaptation to the environment had taken place. The environment was kept constant at 23° C. to 24° C. and at 55 per cent humidity. There was some degree of correlation, in a qualitative sense, between surface temperature and skin flow responses.

We are aware of the fact that our group is small. However, in 6 of the 7 skin-positive subjects, skin blood flow unequivocally decreased. As soon as larger numbers are available, separation in the various groups and statistical evaluation will be done.



F = Flow rate; V = Volume; P = Perfusion rate; L = Leg; F = Foot; S = Skin; M = Muscle; and C = Correction factor

$$F_L + F_F = F$$
$$V_L + V_F = V(\text{cc.})$$
$$P = \frac{F}{V} = \frac{V_L}{V}P_L + \frac{V_F}{V}P_F \text{ (cc./100 cc./min.)}$$

assume

$$V_{LS} = 3V_{LM} \quad \text{and} \quad V_{FS} = 5V_{FM}$$
$$P_M = P_L = \frac{F - F_F}{V - V_F} \quad \text{and} \quad P_S = P_F = \frac{F_F}{V_F}$$
$$\therefore P'_M = P_{LM} = \frac{F_{LM}}{V_{LM}} = \frac{F_L - F_{LS}}{V_L - V_{LS}} = \left(\frac{1 - C_LF}{1 - C_LV}\right)P_M$$
$$P'_S = P_{FS} = \frac{F_{FS}}{V_{FS}} = \frac{F_F - F_{FM}}{V_F = V_{FM}} = \left(\frac{1 - C_FF}{1 - C_FV}\right)P_S$$

FIGURE 1.

TABLE 1.

		Skin flow			Muscle flow		
		Decrease	No change	Increase	Decrease	No change	Increase
Total allergic	7	6	1	0	1	2	4
No OAS	6	5	1	0	1	1	4
OAS	1	1	0	0	0	1	0

TABLE 2.

		Skin flow			Muscle flow		
		Decrease	No change	Increase	Decrease	No change	Increase
Total non-allergic	25	2	15	8	2	12	11
No OAS	19	1	10	8	2	9	8
OAS	6	1	5	0	0	3	3

The following preliminary conclusions seem justified:

1) Vascular responses to tobacco in the lower extremities may be different in skin and muscle; there may be decrease, no change, or increase in flow in varying combinations.

2) Almost every subject whose skin shows positive reactions to tobacco extracts shows a decrease in blood flow after smoking the tobacco to which his skin is sensitive.

3) Most subjects whose skin is not sensitive to tobacco extract have either no change or an increase in skin flow in response to smoking.

4) Changes in muscle flow do not seem to be correlated to skin allergy in any way.

One might, in the light of J. Harold Burn's investigations reported elsewhere in these pages, speculate on the possibility that people with "tobacco-allergy" perhaps handle their catecholamines differently from the nonallergic individual.

References

1. Winterstein, A. & E. Aronson. 1927. Effects of smoking. Z. Hyg. Infectionskrankh. **107:** 487.
2. Maddock, W. G. & F. A. Coller. 1932. Peripheral vasoconstriction by tobacco demonstrated by skin temperature changes. Proc. Soc. Exptl. Biol. Med. **29:** 487–488.
3. Barker, N. W. 1933. Vasoconstrictor effects of smoking. Proc. Staff Meet. Mayo Clin. **8:** 284–287.
4. Abramson, D. I., H. Zazeela & B. S. Oppenheimer. 1939. Plethysmographic studies of peripheral blood flow in man; effect of smoking. Am. Heart J. **18:** 290.
5. Mulinos, M. G. & I. Shulman. 1940. The effects of cigarette smoking and deep breathing on the peripheral vascular system studied by five methods. Am. J. Med. Sci. **199:** 780.
6. Sulzberger, M. B. 1933. Recent immunologic experiments in tobacco hypersensitivity. Bull. N. Y. Acad. Med. **9:** 294.
7. Harkavy, J., S. Hebald & S. Silbert. 1932. Tobacco sensitiveness in thromboangiitis obliterans. Proc. Soc. Exptl. Biol. Med. **30:** 104.
8. Cooke, R. A. 1947. Allergy in Theory and Practice. : 517. Saunders. Philadelphia, Pa.

Part IV. Action of Nicotine and Smoking on Coronary Circulation

PHYSIOLOGY OF THE CORONARY CIRCULATION

Donald E. Gregg
Department of Cardiorespiratory Diseases, Walter Reed Army Institute of Research, Washington, D. C.

I propose to discuss first two considerations that are basic to an understanding of coronary function. These are the control of coronary flow and the control of oxygen usage of the heart of the open-chest dog during systole, when it is contracting, as compared with that during diastole, when it is relaxed. Then I shall discuss a general pattern of response of the coronary circulation to various stress states when the measurements are made in the recumbent dog or human in the anesthetized or unanesthetized condition. Finally, I shall introduce data on coronary flow taken in the unanesthetized and strongly exercising dog. This last consideration should throw some light on the coronary flow response to the stimuli of every day activity and living.

The first consideration is the separation and quantitation of the determinants of coronary flow in the myocardium. This has been most difficult. It involves the separation of the vasomotor action of the small mass of intrinsic smooth muscle built into the coronary blood vessels, which is effective throughout a cardiac cycle, from the mechanical effect exerted only during ventricular systole by the large muscle mass around the coronary vessels. This fractionation is of extreme importance since, under normal conditions, the only other major factor affecting coronary flow is central coronary or aortic pressure, which does not usually change greatly even in exercise. Actually, it is relatively ineffective in controlling coronary flow. It is estimated that doubling the aortic blood pressure might double the coronary flow, but doubling the average radius of the coronary bed increases coronary flow about 16 times (FIGURE 1).

Recently a technique has become available for separation and quantitation of the mechanical factors governing coronary flow from those factors having a direct action on the smooth muscle of the coronary bed. In the open-chest dog, the left coronary artery is perfused with blood under a constant pressure approximating the mean aortic pressure, and the left coronary inflow is quantitated by a rotameter. Continuous measurements are made first in the beating heart, and then are made during ventricular asystole, induced either by peripheral vagal stimulation in the neck or by disconnecting an external pacemaker driving the ventricles, in which complete arteriovenous (AV) heart block had been surgically produced earlier in the experimental day. In both cases the mechanical effects of ventricular contraction are largely removed.[1]

FIGURE 2 illustrates that induction of ventricular asystole by vagal stimulation always increases immediately (within 1 sec.) left coronary inflow, in this instance from 123 to 150 cc./min., and also increases the coronary sinus flow after a 1 to 2 sec. delay. Thus ventricular contraction acts to impede coronary flow through the ventricular wall. The extent of the rise of flow is taken to represent the magnitude of the mechanical or passive factors limiting coronary flow. The magnitude of this mechanical throttling effect on coronary flow dur-

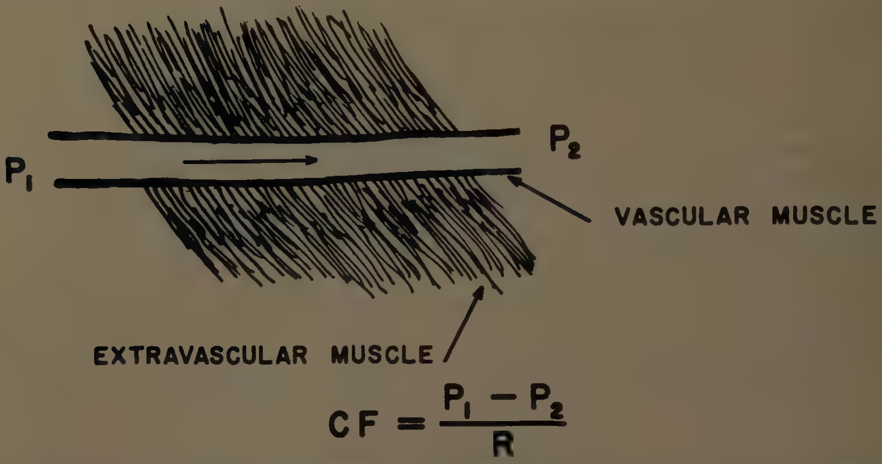


FIGURE 1. Determinants of coronary flow, in which the tube represents the mean coronary bed with its contained smooth muscle; P_1-P_2 is the pressure drop across the coronary bed; R is the sum of the resistances imposed by the intravascular smooth muscle and the myocardial mass.

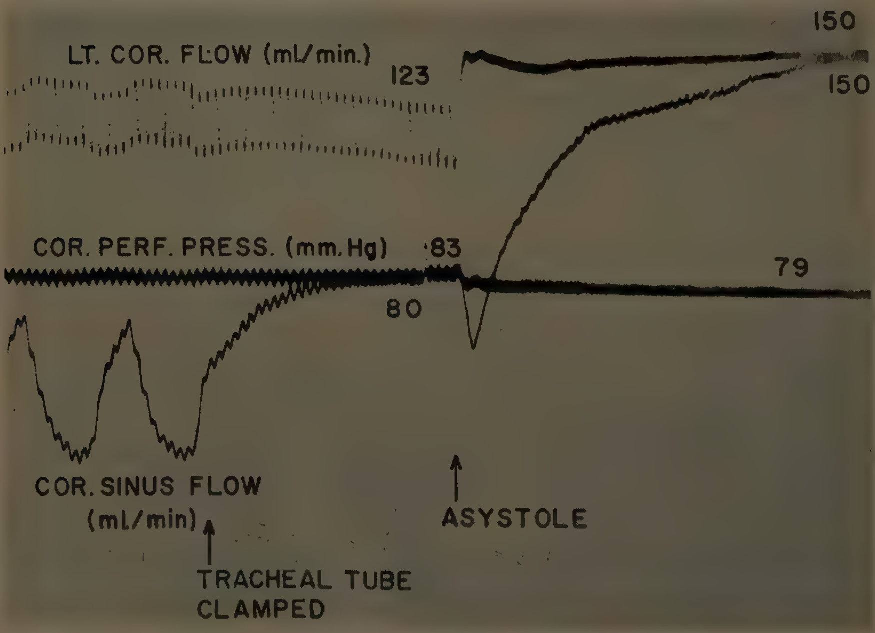


FIGURE 2. Effect of vagal cardiac arrest for 12 sec. on the left coronary inflow and coronary sinus flow when the coronary artery was perfused at a constant pressure of 83 mm. Hg.[1]

ing systole normally varies from 31 to 300 per cent and averages about 50 per cent. The new flow level represents that state of coronary dilatation related to the condition of the intrinsic smooth muscle of the coronary vessels at the prevailing coronary pressure.[2]

TABLE 1 illustrates the relative contribution of extravascular and intravascular resistance to an increase of coronary flow under different conditions. With increased heart rate, extravascular resistance rises but intravascular resistance falls to a greater extent, so that there is a decrease in net coronary resistance. Following a decrease in arterial blood oxygen saturation, coronary flow rises as a result of a decrease in both extra- and intravascular resistance, but even here only 40 per cent of the flow increase comes from a decreased extravascular resistance. With aortic constriction, transfusion, and intracoronary injection of epinephrine, norepinephrine and isoproterenol, there is no change in extravascular resistance, but intravascular resistance decreases considerably. Thus, in these instances the major portion of a flow increase is through active vessel dilatation and not through reduction in extravascular resistance.[2,3,4]

TABLE 1

EFFECT OF DIFFERENT VARIABLES ON THE FRACTIONATION OF CONTROL OF CORONARY FLOW BY INTRAVASCULAR AND EXTRAVASCULAR MUSCLE

	Extravascular contribution to coronary flow increase	Intravascular contribution to coronary flow increase
↗Heart rate (90 to 240)	−100%	+200%
↘Arterial blood oxygen saturation	+40%	+60%
↗Arterial blood pressure by aortic clamp	0%	+100%
↗Venous return by infusion	0%	+100%
Epinephrine, norepinephrine, isoproterenol (intra cor)	0%	+100%

The second consideration is the identification of the determinants of myocardial oxygen usage. This is also most difficult, and our knowledge is sufficient only to permit discussion of the subject. We know that the myocardium takes up oxygen during both systole and diastole. With our presently poor methodology, separation can be made only by measuring oxygen uptake, first in the beating heart during repetitive systoles and diastoles, and then in the relaxed heart or during prolonged diastole and, finally, obtaining the oxygen usage during systole by difference.

Estimation of the metabolism of the myocardium in the absence of a heart beat, that is, during prolonged diastole, has been made in the vagal arrested heart as previously described. The oxygen saturation of the arterial blood and coronary sinus blood was also measured continuously. This permitted left coronary arteriovenous oxygen difference as well as coronary inflow to be measured continuously, first in the beating heart, and then in the stopped heart, until a new equilibrium had been established, usually within 20 to 25 sec.

Typical results are shown in FIGURE 3. As coronary inflow rises immediately with asystole from 70 to 90 cc./min., the oxygen saturation of blood in the coronary sinus also rises from a low control value of 44 per cent to a very high value of 81 per cent, thus greatly reducing the coronary arteriovenous oxygen difference.

Calculations in many experiments show that as the result of the combination of increased coronary flow and decreased coronary arteriovenous oxygen difference, the average oxygen usage/100 gm. left ventricle/min. decreases from the average control level of 8.1 ml. in the beating heart to 2.3 ml. in the resting heart, or to 30 per cent of the control (TABLE 2). Individual values range from 1.0 to 4.0 ml. This oxygen consumption in diastole is about one third that in systole for an equivalent time period.

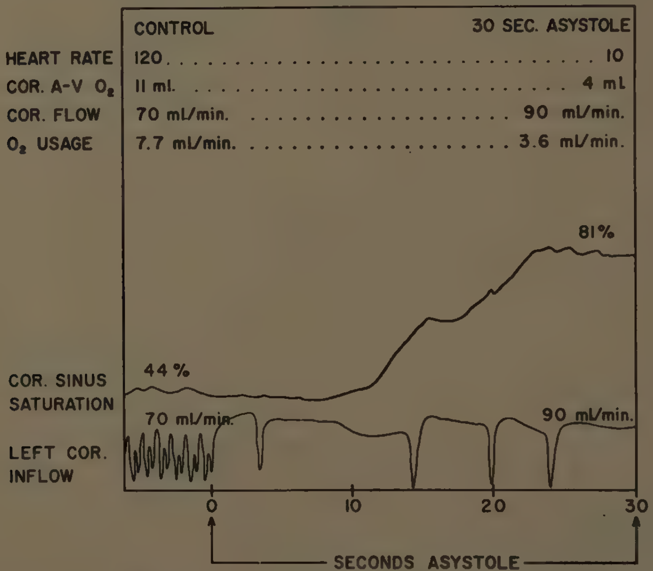


FIGURE 3. Effect of vagal cardiac arrest on left coronary inflow and coronary sinus oxygen saturation while the coronary artery was perfused at a constant pressure. Four heart beats occurred during the 30 sec. of arrest.[3]

TABLE 2

OXYGEN USAGE OF THE LEFT VENTRICLE BEFORE AND DURING STOPPAGE OF ITS EXTERNAL WORK BY FOUR PROCEDURES

(cc. O_2/100 gm. Left ventricle per min.)

	Before	During	%
Vagus	8.1	2.3	30
Potassium	7.7	2.0	27
Hemorrhage	8.8	3.4	40
Ventricular fibrillation	8.0	3.8	47

Attention is also directed to the values for oxygen usage obtained in the same type of preparation but in which the external work of the heart was reduced to zero by other means. In the potassium-stopped heart, the oxygen usage of 2 cc. during diastole is about the same. In the beating heart emptied by suction and hemorrhage and in the fibrillating heart, the oxygen usages of 3.4 and 3.8 cc. are much greater.

These oxygen values have some interesting characteristics. The oxygen uptake during vagal arrest or removal of the artificial pacemaker is unaffected by the ventricular diastolic size. In any one experiment, the value is not fixed but varies greatly with the control metabolic level in the beating heart. With intracoronary artery epinephrine or norepinephrine injection, the values are especially high and may equal 50 per cent of those in the control state.

Attention is directed to at least one implication of such experiments. As in any other muscle, the mechanical efficiency of the heart is estimated by dividing the external work of the ventricle by the difference between the energy equivalent of its oxygen usage while beating and at rest. If the experimentally measured oxygen value of 2.3 ml./min. approximates the resting metabolism of 100 gm. of left ventricle, while the oxygen uptake in the beating heart approximates 8.1 ml., then calculation of ventricular efficiency without considering the diastolic metabolism is in serious error. For example, if a cardiac efficiency without such correction is 15 per cent, then inclusion of the correction would increase the calculated efficiency from 15 to 21 per cent (TABLE 3).[5]

TABLE 3

CALCULATION OF VENTRICULAR EFFICIENCIES, TAKING INTO ACCOUNT THE OXYGEN USED WHEN THE HEART WAS DOING NO EXTERNAL WORK

Effect of Resting O_2 Usage on Calculation of Cardiac Efficiency

$$\text{Efficiency} = \frac{\text{Left ventricular (kg.-m./min.) (100)}}{(\text{Total left ventricular } O_2 \text{ used} - \text{resting } O_2 \text{ used})\ 2.057}$$

$$\text{Efficiency} = \frac{1.2}{8.1}(100) = 15\%$$

$$\text{Efficiency} = \frac{1.2}{8.1 - 2.3}(100) = 22\%$$

$$\text{Efficiency} = \frac{1.2}{8.1 - 3.4}(100) = 26\%$$

Adjustment of the mechanical efficiency of the left ventricle by this amount may not be sufficient. Instead of using vagal stimulation to stop the external cardiac work, one can empty the heart rapidly by hemorrhage and suction and thus obtain a nonworking but empty heart. Here the average oxygen usage, 3.4 ml./100 gm./min., is considerably greater than that with vagal asystole. Such values are often still higher, since they vary not only with the preceding level of metabolism of the heart beating and doing external work, but also with the prevailing frequency in the empty heart. Correction of the mechanical efficiency of the left ventricle on this basis obviously gives a still higher efficiency (26 per cent).

For reasons such as these, then, until the oxygen usage of the myocardium doing no external work is known under various physiological conditions, calculation of or reference to cardiac efficiency would seem to be premature.

The preceding gives some idea of the fractionation of the control of coronary flow and oxygen uptake within the myocardium during a cardiac cycle. The third consideration is concerned with the over-all control of the coronary circulation. There are actually certain general patterns of hemodynamic responses of the heart and coronary circulation which follow primary changes in stress. The information has been obtained from both humans and animals and has been assembled from many laboratories (FIGURE 4). With increased arterial CO_2 content or decreased blood *p*H, with short periods of ischemia, mild anemia, and anoxia, there is little or no change in systemic dynamics or myocardial oxygen usage. Except for the first two instances, however, coronary vascular resistance is decreased and oxygen consumption is unchanged as the result of the combination of an increased coronary flow and a decreased coronary arteriovenous oxygen difference. When the primary change in stress is an increased heart rate, increased venous return (whole blood transfusion), increased cardiac work, aortic coarctation (moderate), thyrotoxicosis, cardiac action of fibers from the sympathetic nerves, intravenous injection of epinephrine or norepinephrine, severe anemia or anoxia, there is an increase in cardiac work and cardiac oxygen usage. Concurrently, coronary flow increases while coronary vascular resistance decreases. In situations of primary decrease in stress such as hemorrhagic hypotension, hypothermia, and extreme heart failure, the cardiac work and oxygen consumption decrease, as does the coronary inflow. An exception to this general picture of coronary compensation to changing stress is that of complete aortic coarctation, which may be characterized by a reduction in the output and work of the heart and by an increase in left coronary flow and oxygen usage. A second exception is that of chronic hypertension, in which the cardiac work is increased but cardiac output, oxygen usage, and left coronary flow are unaltered, while coronary resistance to flow increases.[6]

Is there one over-all unifying mechanism controlling coronary flow? Is there another mechanism controlling the oxygen usage of the myocardium? The answers have been sought for many years. Although in most instances coronary flow follows a primary increase or decrease in cardiac stress, that is, cardiac work and cardiac output, there are exceptions, as we have seen. However, correlation of coronary flow with oxygen usage is excellent, the exceptions being ischemia, mild anemia, and anoxia. This relationship is understandable since the oxygen consumption is derived from the product of the coronary flow and the coronary arteriovenous oxygen difference. Normally, most oxygen is removed from the coronary blood and the level of coronary sinus oxygen is usually fairly constant under stress; that is, it does not change more than 10 to 15 per cent. In those instances in which the coronary arteriovenous oxygen difference increases or decreases by this amount, it does not greatly affect the relation of coronary flow to oxygen usage since the change is very small relative to the magnitude of the coronary flow change.

It would be expected that the oxygen consumption during systole, when ventricular muscle is shortening and developing tension, would depend in some way on the dynamic adaptation of the ventricle to its work. The presence of some such correlation was first illustrated by the experiments of Evans and Matsuoka,[7] and of Starling and Visscher,[8] indicating in the isolated heart a linear relation between diastolic volume and oxygen usage. More recently,[9] it has been

CIRCULATORY RESPONSES TO STRESS STATES

CONDITION	Cardiac Work	Cardiac Output	Coronary Flow	$\frac{\text{Cor. Press.}}{\text{Cor. Flow}}$	Oxygen Usage	Coronary a-v O_2
↗ Blood CO_2, ↘ blood *p*H	→	→	→	→	→	→
Ischemia, mild anemia and anoxia	→	→	↗	↘	→	↘
↗ Heart rate, ↗ CO (transfusion) ↗ C.W. Aortic coarctat. (mild)[1], thyrotoxicosis Cardiac sympathetic nerves, epinephrine and norepinephrine I.V. Severe anemia[2] and anoxia[2]	↗	↗	↗	↘	↗	→
Hypothermia, shock (hemorrhagic) Heart Failure (extreme)[3]	↘	↘	↘		↘	→
Aortic coarctation (complete)	↘	↘	↗	↘	↗	→
Hypertensive cardiovascular disease	↗	→	→	↗	→	→

[1] Cardiac output → or ↘ [2] Cor. a-v O_2 ↘ [3] Cor. a-v O_2 ↗

FIGURE 4. Circulatory responses to various states of stress.

found that oxygen usage correlates best with the total tension developed by the left ventricle. It is believed, however, that final decision as to whether any of these determinants of oxygen usage is primary or empirical must await the necessary measurements under normal conditions without anesthesia or surgical insult.

In closing, I add a word of caution. Most of the information we have considered is based upon observations in the open-chest dog under stress states. It is not known to what extent it applies to normal situations, since it has been obtained under conditions far removed from normal as the result of insults from anesthesia, surgery, and trauma. It is true that semiquantitative values for coronary flow and oxygen usage are available in the resting human and dog. No information exists, however, in normal humans and animals, as to the regulation of the coronary circulation exposed to the stresses of every day life, such as exercise and excitement.

A start is being made in alleviating this situation. Successful direct measurements of coronary flow have been made in normal unanesthetized dogs during the last year. Some of these measurements have been made by applying chronically an appropriate electromagnetic flow meter (a somewhat modified Kolin type) directly to the circumflex branch of the left coronary artery. Although this gives correct flow values, very often it causes thrombosis within a few days because of the violent heart action. To remove the flow probe to a quiet area, R. J. Hall, of our laboratory, has anastomosed the left internal mammary artery to the left coronary artery of dogs, using a nonsuture technique so that there is intimal to intimal contact (FIGURE 5). The anastomoses in 22 of 25 such dogs have been demonstrated to be patent and without myocardial infarction up to the time of sacrifice, which was 12 to 18 months after operation. This is thus a standardizable technique.

Its use is shown in the last figure. Illustrated in FIGURE 6 are the changes in phasic coronary flow in such a dog 15 months after construction of the anastomosis and 7 weeks after implantation of the electromagnetic flow meter probe. First, the dog stood on a treadmill, then ran for 3 min. at 12 mph at a 5 per cent grade and, finally, he stood at rest. Although not measured, the blood pressure and cardiac output obviously rose in this dog as they have in other dogs. A pulse pressure pattern has been sketched in for orientation purposes. The control flow is 46 cc./min., most of this occurring during diastole. Within 15 to 20 sec. after the onset of exercise, the changes in the coronary flow response are complete and do not change further during the 3-min. period of exercise. The changes include an increase in heart rate from 80 to 220/min., a reduction in duration of both systole and diastole per beat, an increase in mean flow from 46 to 111 cc./min., as the result of a large reduction in systolic flow and an increase in diastolic flow per beat, and accentuation of back flow during systole. Calculation shows that the flow per heart beat is mildly decreased; hence the mean flow increase is entirely on the basis of the increase in heart rate. Within 5 sec. after sudden stoppage of the treadmill, the patterns and flow have started to revert toward normal, and the return to normal is completed within about 7 min. The last record was taken so that a comparison could be made between the maximum diastolic rate of flow during exercise and that during reactive hyperemia. It was taken shortly after the 7-min. recovery period and immediately following a 5-sec. occlusion, by a pneumatic cuff, of the coronary artery in which flow was being measured. Despite the slower heart rate, maximum diastolic flow during reactive hyperemia is about the same as that during exercise.

The control coronary flow patterns in these dogs are generally similar to those obtained many years ago in the open-chest dog in which coronary flow was measured with an orifice meter. As shown here the coronary flow response to exercise is not the response that one observes when the workload of the heart is increased by transfusion or elevation of aortic pressure by aortic constriction. It is similar, however, to a combination of the effects observed resulting from a mechanical increase in heart rate by means of an artificial pacemaker and from the augmentation of cardiac contractility resulting from stimulation of the cardiac fibers from the left stellate ganglion, or following the intracoronary injection of epinephrine or Levophed. Such responses were obtained 15 years ago in the open-chest dog, using an orifice meter to measure the coronary flow.

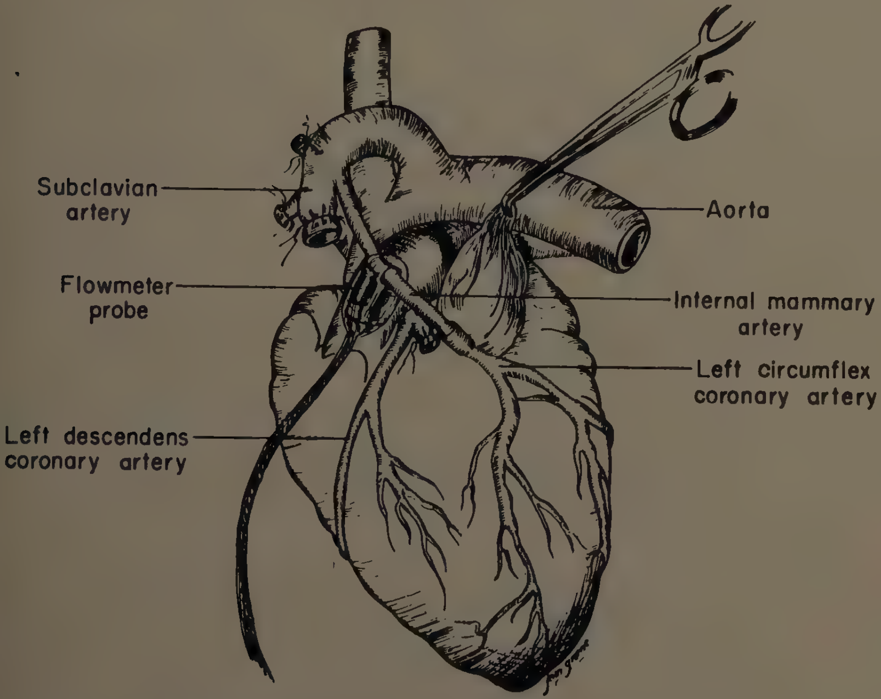

Subclavian artery
Flowmeter probe
Left descendens coronary artery
Aorta
Internal mammary artery
Left circumflex coronary artery

FIGURE 5. Schematic drawing showing anastomosis of internal mammary artery to left circumflex coronary artery.

We are finding this to be a very useful tool for the study of the normal coronary circulation in the active dog. I predict that such studies will lead to some recasting of our present concepts of the control of the coronary circulation.

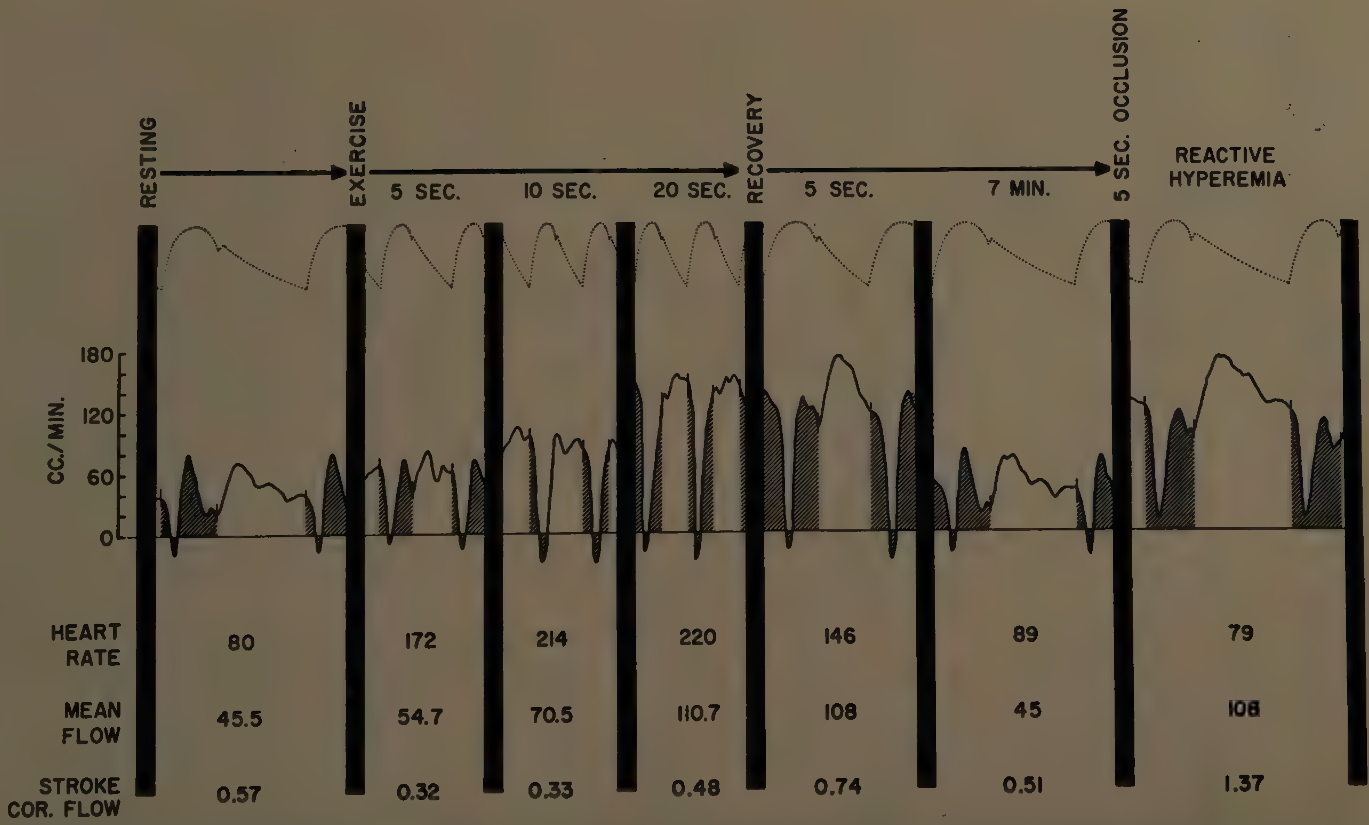


FIGURE 6. Redrawn excerpts from record of coronary flow through left circumflex artery taken in dog running on treadmill at 12 mph on a 5 per cent grade for 3 min. Dog was tested 17 months after anastomosis and 7 weeks after implantation of electromagnetic flow meter probe.

Unfortunately, at present sufficient data has not accumulated to warrant any further statement.[10]

References

1. Sabiston, D. C. & D. E. Gregg. 1957. Effect of cardiac contraction on coronary blood flow. Circulation. **15:** 14.
2. Lewis, F. B., J. D. Coffman & D. E. Gregg. 1959. The effect of heart rate and intra-coronary isoproterenol, levarterenol and epinephrine on coronary flow and resistance. (Abstr.) Federation Proc. **18:** 91.
3. Gregg, D. E. 1955. Some problems of the coronary circulation. Verhandlunger der Deutschen Gesellschaft für Kreislaufforschung. **21:** 22.
4. Gregg, D. E. 1958. Regulation of the collateral and coronary circulation of the heart. Circulation. **:** 163–186. Proceedings of Harvey Tercentenary Congress. Blackwell Scientific Publ. Oxford, England.
5. McKeever, W. P., D. E. Gregg & P. C. Canney. 1958. Oxygen uptake of the non-working left ventricle. Circulation Research. **6:** 612–623.
6. Gregg, D. E. 1959. Coronary Circulation. Encyclopedia of Cardiology. **1:** Chapt. 23. McGraw-Hill-Blakiston. New York, N. Y.
7. Evans, C. L. & Y. Matsuoka. 1915. The effect of various mechanical conditions on the gaseous metabolism and efficiency of the mammalian heart. J. Physiol. London. **49:** 378.
8. Starling, E. H. & M. B. Visscher. 1927. The regulation of the energy output of the heart. J. Physiol. London. **62:** 243.
9. Sarnoff, S. J., E. Braunwald, G. H. Welch, Jr., R. B. Case, W. N. Stainsby & R. Macruz. 1958. Hemodynamic determinants of oxygen consumption of the heart with special reference to the tension-time index. Am. J. Physiol. **192:** 148.
10. Hall, R. J., E. M. Khouri & D. E. Gregg. 1959. Nonsuture internal mammary—coronary artery anastomosis. Proc. 32nd Scientific Sessions, Am. Heart Assoc. **:** 791

CARDIAC EFFECTS OF INTRACORONARY ARTERIAL INJECTIONS OF NICOTINE*

Samuel Bellet, James W. West, Santiago V. Guzman
Department of Pharmacology, School of Medicine, University of Pennsylvania, and the Division of Cardiology, Philadelphia General Hospital, both in Philadelphia, Pa.

The purpose of this study was to determine the cardiac effects of nicotine by direct coronary artery injection. This method has the advantage of elucidating certain direct cardiac effects independent of systemic changes. The procedure previously described from this laboratory involves the ability to inject minute amounts of drugs or dye into various coronary branches of dogs without opening their chests or interfering with perivascular nerves.[1,3]

Methods and Material

The experiments were performed on 33 normal adult mongrel dogs, weighing 20 to 30 kg. each, under light anesthesia by pentobarbital, 30 mg./kg., intravenously. Systemic blood pressure was recorded from the femoral artery using a Sanborn electromanometer and a direct-writing Sanborn polyviso recorder. The electrocardiogram was continuously monitored in the same recorder using a precordial lead V_3. In some experiments, myocardial contractility was measured by means of a Walton strain gauge sutured directly on the exposed surface of the ventricle. The cardiomyogram was recorded in the same multichanneled Sanborn polyviso recorder. Coronary blood flow was measured as outflow from the coronary sinus in the intact animal by a cannula passed through the external jugular vein into the coronary sinus.

Catheterization of coronary sinus was accomplished in the intact animal by a special coronary sinus catheter inserted by way of the external jugular vein under fluoroscopic guidance. The catheter was provided with multiple openings at the tip and an inflatable balloon for securing it in the coronary sinus. Catheterization of the main branches of the coronary arterial tree, right, left, and anterior descending and left circumflex branches was accomplished under fluoroscopic guidance. The chest was intact in all experiments, except those involving the Walton myocardiograph.

Results

Effects on myocardial contractility. A total of 86 observations were made on 15 dogs. In most cases the strain gauge was attached to the anterior surface of the left ventricle and nicotine was injected into the left anterior descending branch. In all 15 dogs, injections of nicotine ranging from 0.2 to 2.2 μg./kg. resulted in an increase in contractility ranging from 40 to 90 per cent, with an average increase of 75 per cent. A similar increase in contractility was also observed in the right ventricle and in the posterior aspect of the left ventricle. When nicotine was injected into the corresponding artery, the right coronary

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* The work described in this paper was supported in part by research grants from the Tobacco Industry Research Committee and from the Life Insurance Medical Research Fund, both in New York, N. Y.

and the left circumflex respectively, the increase in force of contraction was accompanied by electrocardiographic changes consisting of S-T depression and T-wave inversion (FIGURE 1). An increase in systemic blood pressure was also seen occasionally. Tetraethylammonium (TEA), when injected into the

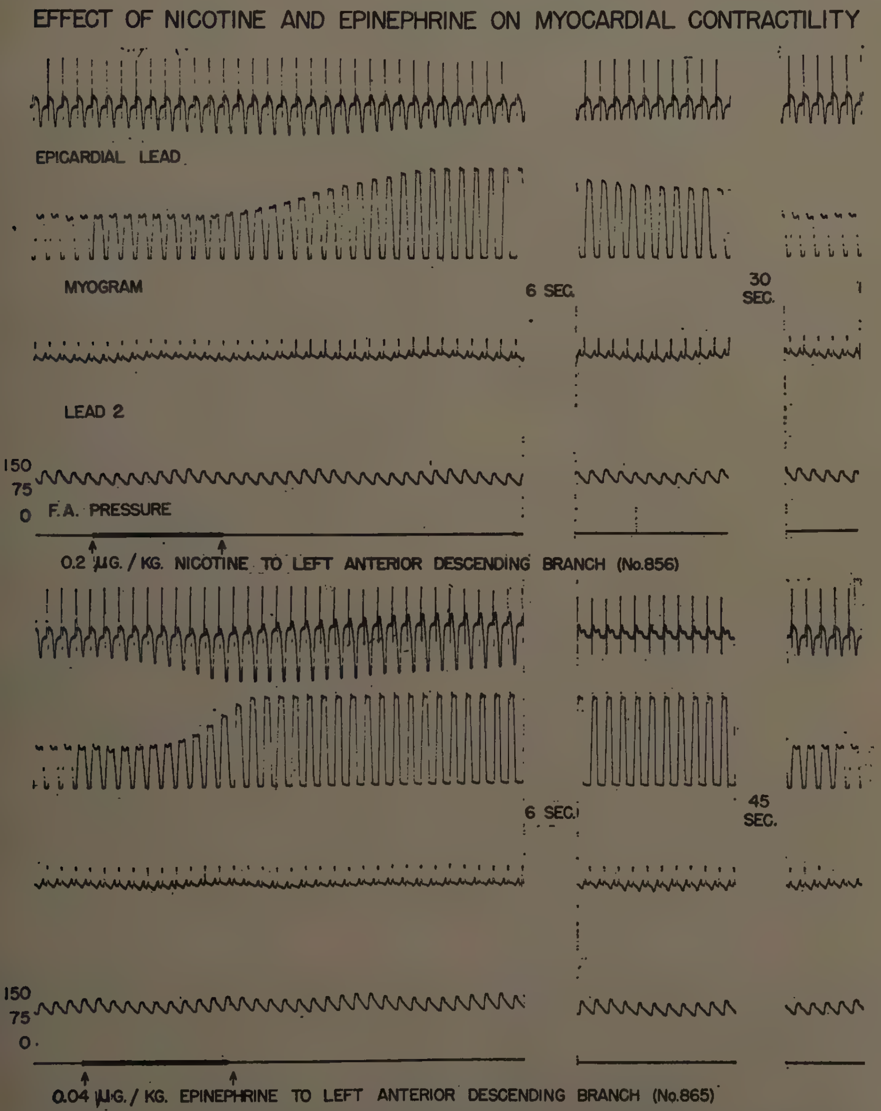

FIGURE 1. Effects of nicotine and epinephrine injected into left anterior descending branch. The records (from *top*) taken from two different experiments show: epicardial lead, myogram (strain gauge attached to the anterior surface of the left ventricle), lead 2, and femoral arterial blood pressure. Note the marked increase (*top*) in the height of contraction accompanied by S-T wave changes in the electrocardiogram following nicotine injection. *Bottom* shows that these effects of nicotine are essentially similar to the positive inotropic and electrocardiographic effects of epinephrine when injected into the same site. Speed of recorder is 25 mm./sec. Reproduced by permission of *Circulation Research.*

same site, completely prevented the stimulating action of nicotine on myocardial contractility, as well as the accompanying electrocardiographic effects.

Right coronary artery. Nicotine, when injected into the main right coronary artery in 6 dogs, produced transient periods of sinus arrest, sinus bradycardia, atrial fibrillation, and occasional sinus tachycardia.

Left anterior descending branch. Small doses of nicotine produced only S-T segment depression or S-T depression and inversion of the T waves.

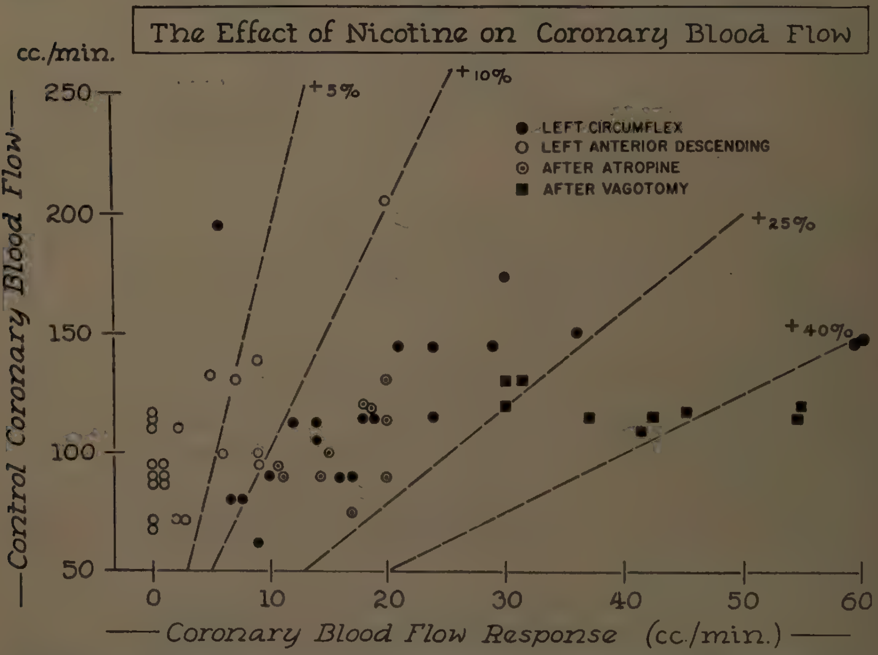

FIGURE 2. Coronary sinus blood flow after coronary arterial injections of nicotine. The responses plotted in the graph represent the peak changes (increases) observed immediately after coronary arterial injections of nicotine. Left anterior descending injections prior to atropine or vagotomy produced essentially no increase in coronary flow; however, after atropine or vagotomy, left anterior descending injections increased coronary artery flow. Left circumflex injections increased coronary artery blood flow. Heart rate and blood pressure remained constant during these effects of nicotine. Reproduced by permission of *Circulation Research.*

Left circumflex branch. Nicotine produced S-T and T-wave changes and transient periods of complete AV heart block, with temporary cessation of ventricular beats followed by nodal escape.

Bezold-Jarisch effect. Larger doses of nicotine (1.2 to 2 µg./kg.), when injected into the anterior descending or circumflex branch of the left coronary artery, produced transient periods of sinus arrest or sinus bradycardia, with or without AV heart block. These effects were accompanied by hypotension.

Blocking procedures. Bilateral vagotomy completely abolished the Bezold-Jarisch effects resulting from left coronary arterial injection of nicotine. However, the AV block accompanying left circumflex injection still persisted.

Atropine abolished all cardiac inhibition produced by nicotine from right and left coronary arterial injection, but did not prevent the concomitant hypotension. TEA completely blocked all the effects of intracoronary injection of nicotine.

Effects on coronary blood flow. In no case was there any sign of direct coronary vasoconstriction by nicotine. In 5 dogs, following left circumflex injection, there was an increase from 8 to 42 per cent in the coronary blood flow, with an average increase of 19 per cent. Following injection to the left anterior descending branch, there was essentially no change in coronary blood flow (FIGURE 2).

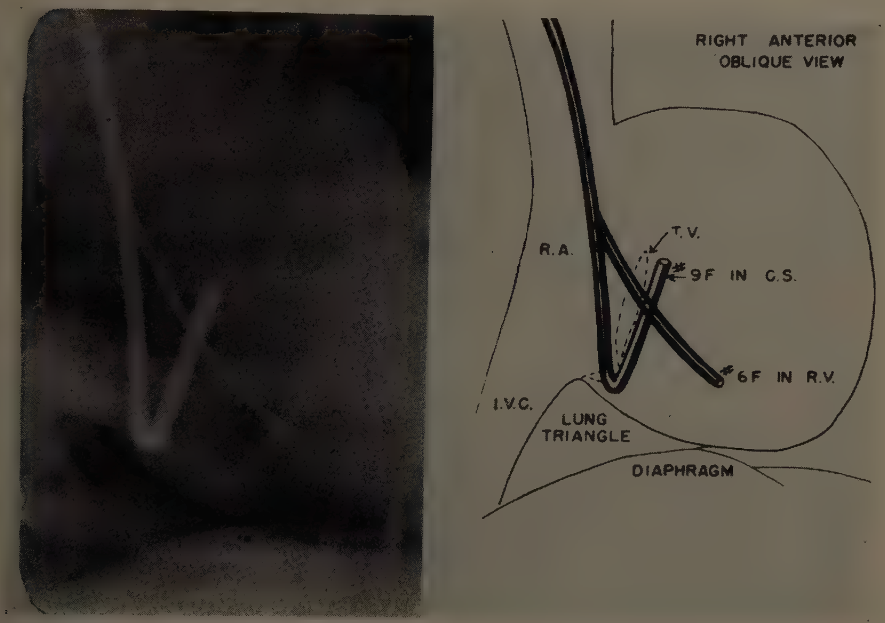

FIGURE 3. A catheter in the coronary sinus (C.S.) with another catheter in the right ventricle (R.V.) is illustrated in the photograph. The picture on the left was obtained from an X ray; sketch of the X ray is seen on the right. Dog is placed in the right anterior oblique position.

There was no evidence that any dose of nicotine injected into the three main coronary branches produced constriction of the coronary arteries. Measurement of coronary sinus outflow showed a decrease only when the blood pressure fell as a result of marked ventricular slowing (FIGURE 3). Positive chronotropic and inotropic effects were accompanied by increases in coronary blood flow.

Summary

Intracoronary arterial injections of nicotine in lightly anesthetized dogs elicited parasympathetic, sympathomimetic, and Bezold-Jarisch responses. The nature of these responses are discussed above.

Tetraethylammonium chloride completely blocked the effects of nicotine on myocardial contractility, coronary blood flow, electrocardiogram, and blood pressure. Atropine abolished all the cardiac inhibition induced by nicotine from the right and left coronary arterial injections, but did not prevent the change in blood pressure (hypotension) elicited from left coronary artery injections. Bilateral vagotomy completely abolished the Bezold-Jarisch effects resulting from left coronary arterial injections of nicotine, but did not prevent the effects of injection into the right coronary and left circumflex branches of the S-A and AV nodes, respectively. There was no evidence of coronary vasoconstriction as indicated by a decrease in coronary blood flow following intracoronary arterial injections of nicotine.

The results indicate that chemoreceptors, parasympathetic and sympathetic, or sympathetic-like ganglia (chromaffin tissue) affected by nicotine, are present in the heart, and that the type of response depends on the predominant ganglia or receptor stimulated at the site of injection.

Acknowledgments

We thank the American Heart Association, New York, N. Y., and *Circulation Research* for permission to reproduce FIGURES 1 and 2 and other previously published material (1958).

References

1. WEST, J., T. KOBAYASHI & S. V. GUZMAN. 1958. Coronary artery catheterization in the intact dog. Circulation Research. **6:** 383.
2. WEST, J., S. V. GUZMAN & S. BELLET. 1958. Cardiac effects of intracoronary arterial injections of nicotine. Circulation Research. **6:** 389.

ACTION OF NICOTINE AND SMOKING ON CORONARY CIRCULATION AND MYOCARDIAL OXYGEN UTILIZATION

Gerald A. Kien and Theodore R. Sherrod
Department of Pharmacology, University of Illinois, College of Medicine, Chicago, Ill.

Introduction

The alterations of the electrocardiogram (von Ahn, 1954; Russek *et al.*, 1955) and the ballistocardiogram (Davis *et al.*, 1953, 1956; Henderson, 1953) that follow inhalation of cigarette smoke or administration of nicotine have generally been thought to result as a consequence of either coronary artery constriction or an increase in the work of the heart beyond the capacity of the coronary arteries to supply the necessary metabolic requirements of the myocardium. It has been adequately demonstrated, however, that cigarette smoke inhalation by the normal person results in a significantly increased coronary blood flow with no significant increase in myocardial substrate or oxygen utilization (Bargerson *et al.*, 1957). An increase in coronary blood flow following administration of cigarette smoke or nicotine has also been demonstrated in the intact dog (Kien *et al.*, 1957, 1958; Schmitthenner *et al.*, 1956), in the heart-lung preparation (Mansfield and Hecht, 1933), and in the isolated perfused rabbit heart (Laubry *et al.*, 1933). These findings fail to support the concept that nicotine administration or cigarette smoke inhalation causes coronary artery constriction in the normal heart. It has been shown, however, that while nicotine caused an increase in coronary flow in the normal isolated perfused rabbit heart, it caused a marked diminution of coronary flow in the atherosclerotic rabbit heart (Rinzer *et al.*, 1956).

Administration of nicotine or cigarette smoke to the open chest anesthetized dog results in an increase in blood pressure. Cardiac output and heart rate changes were found to be variable; however, calculations based on these measurements revealed a consistent increase in the level of cardiac work (Kien *et al.*, 1958). The inability of the coronary arteries to dilate sufficiently to supply the increased metabolic requirements of the myocardium as a consequence of its increased workload has been suggested as the cause of the adverse electrocardiographic manifestations of cigarette smoke inhalation or nicotine administration. It has been demonstrated (Bellet *et al.*, 1941) that in dogs with ligated coronary arteries the amount of nicotine necessary to elicit electrocardiographic alterations was markedly less than that required to elicit these changes in the normal dog. When the ability of the coronary arteries to compensate for an increased workload is impaired, such as in the atherosclerotic heart (Rinzer *et al.*, 1956), or in the heart with ligated coronary arteries (Bellet *et al.*, 1941), administration of cigarette smoke or nicotine elicits an adverse change in the dynamics and in the electrical activity of the myocardium.

Earlier investigations revealed that administration of cigarette smoke or nicotine to the open-chest anesthetized dog resulted in increases in the cardiac workload and in myocardial oxygen utilization (Kien *et al.*, 1958). The temporal occurrence and the severity of the electrocardiographic manifestations did not appear to be related to the changes in cardiac work. In several cases, marked electrocardiographic alterations occurred in the absence of changes in cardiac work and, in contrast, pronounced changes in cardiac work sometimes occurred in the absence of electrocardiographic changes. Although the increase in cardiac work may contribute to the severity of the cardiac effects of cigarette smoke or nicotine administration, it is doubtful that this is the sole contributing factor.

This communication deals with a quantitative evaluation of the relationship between the electrocardiographic alterations and changes in cardiac dynamics following administration of a controlled dose of cigarette smoke, nicotine or epinephrine to the open-chest anesthetized dog.

Methods

Dogs weighing 12 to 20 kg. were anesthetized by intraperitoneal administration of pentobarbital sodium (30 mg./kg. body weight). A tracheal cannula was inserted and the dog maintained on room air by means of a positive pressure respirator. A metal sound of small diameter was inserted into the aorta by way of a corotid artery and blood pressure was measured by means of a pressure transducer.

Coronary blood flow was measured by means of a Shipley rotameter (Shipley and Wilson, 1951) which was interposed between a femoral artery and the circumflex branch of the left coronary artery. Preparation of the animal for this measurement was as follows: A thoracotomy was performed at the fourth intercostal space. The pericardium was incised over the left artrium and the auricular appendage was retracted from the field. An incision was made in the epicardium over the circumflex branch of the left coronary artery immediately distal to its bifurcation with the anterior descending branch. A 1-cm. section of the artery was freed and cannulated with polyethylene tubing, to which was connected the outflow arm of the rotameter. Heparin sodium (10 mg./kg. body weight) was used as the anticoagulant. Arterial blood was supplied to the rotameter from a femoral artery. Cannulation was usually accomplished within 1 or 2 min. after ligation of the coronary artery.

Blood pressure, coronary flow, and the electrocardiogram were simultaneously recorded. Cardiac output was determined by the direct Fick procedure.

Cardiac work was determined by making use of the following formula (Gregg, 1950):

$$W = QR + \frac{mV^2}{2g},$$

where W = work of the left ventricle in gram-meters per beat, Q = mean cardiac output in cubic centimeters per beat, R = mean aortic pressure in meters of mercury, m = mass of blood ejected in grams, and g = gravity factor, 9.8 meters per second per second.

The mean velocity of the blood during systolic ejection was determined by means of the equation:

$$V = \frac{Q}{Ad}$$

where V = velocity of the blood at the base of the aorta in centimeters per second, d = duration of systolic ejection in seconds, and A = cross-section area of the base of the aorta in square millimeters.

Cardiac oxygen consumption per minute was measured as the product of the coronary blood flow per minute and the cardiac arterio-venous oxygen difference. Coronary venous blood samples were obtained through a soft Koroseal catheter, which was inserted into the distal end of the great cardiac vein by way of the external jugular vein, the superior vena cava, and the coronary sinus. The cannula was guided by manipulation of the heart and its position was confirmed at the termination of each experiment. Coronary venous blood was allowed to flow freely from the catheter and was returned to the dog at frequent intervals via a femoral vein. Arterial blood samples were obtained from the thoracic aorta by means of a catheter inserted through a femoral artery. Arterial and venous blood samples of 4 ml. were collected simultaneously under anaerobic conditions in glycerinized glass syringes, to which mercury was added to facilitate mixing of the cells. Mixed venous blood was obtained by direct puncture of the right ventricle. The blood samples were immediately refrigerated until analyzed for blood oxygen according to the method of Van Slyke and Neill (1924).

Cigarette smoke was administered to the dog by placing a standard brand, "king-sized" nonfiltered cigarette in a holder adapted to fit the inflow arm of the positive pressure respirator. The respirator was adjusted to deliver 5 intermittent aliquots of cigarette smoke over a 30-sec. period, with the total quantity of smoke delivered between 1200 and 1500 ml. The cigarette smoke was then discontinued and the dog again respired room air. Nicotine base, in a dose of 20 μg./kg. body weight, and epinephrine chloride in a dose of 5 μg./kg. body weight, were administered intravenously.

In exploration experiments, duplicate measurements of cardiac work and cardiac oxygen utilization were made during the control period. Cigarette smoke or the drugs were administered, and cardiac work and cardiac oxygen utilization were measured only at the onset of the electrocardiographic changes. Statistical analyses were made on the basis of a paired analysis of the control and experimental values obtained at the time of the electrocardiographic alterations. Since there was great variability in the changes in cardiac work and cardiac oxygen utilization, the result of an individual experiment was considered significant if the change due to cigarette smoke or drug administration was greater than two times the variation observed in duplicate control measurements; this value was in excess of two standard deviations of the total variations in the control values.

Subsequent experiments were designed to obtain continuous measurements of these cardiac parameters. Withdrawal of blood samples and measurements of blood pressure, coronary blood flow, and the electrocardiogram were started 1 min. prior to administration of cigarette smoke or the drugs. The measurements were continued throughout the period of drug effect. Coronary venous blood was withdrawn continuously and samples representing 10-sec. intervals were analyzed. For the measurement of cardiac output, consecutive samples of 30-sec. periods were obtained. The blood withdrawn was replaced with an equal quantity of heparinized blood from a donor dog.

Results

Our earlier investigations have demonstrated that the electrocardiographic alterations subsequent to administration of cigarette smoke or nicotine could not be correlated, either temporally or in magnitude, with the workload imposed on the heart. TABLES 1, 2, and 3 represent the changes in cardiac work

TABLE 1

THE EFFECT OF CIGARETTE SMOKE (1200 TO 1500 ML.) ON THE DYNAMICS AND OXYGEN UTILIZATION OF THE HEART DURING MAXIMAL ELECTROCARDIOGRAPHIC ALTERATIONS

Exp. No.	Cardiac work		Oxygen usage		Blood pressure		Cardiac output		Coronary flow		Heart rate	
	Control	Change	Control	Change	Control	Change	Control	Change	Control	Change	Control	Change
	kg.-m./min.		ml./min.		mm. Hg		ml./min.		ml./min.		beats/min.	
1	1.43	+3.55*	6.48	+0.66*	67	+46	1347	+1685	65	+11	150	+50
2	1.77	+0.11	4.39	+1.47*	53	+4	2417	−36	44	+12	80	0
3	0.90	+6.05*	4.07	+2.19*	33	+68	1966	+2489	36	+26	138	−12
4	0.67	+0.02	0.68	+0.53*	49	+6	789	−188	17	+5	156	−15
5	0.81	+1.71*	0.95	+0.99*	68	+10	847	+1000	17	+14	110	+40
6	0.69	+0.02	1.45	+0.20	41	+9	1149	−20	23	+9	132	−20
7	0.37	+0.76*	1.75	+1.95*	42	+20	636	+650	21	+23	120	−50
8	0.36	+0.19	0.52	+0.13	34	+10	845	+290	23	+3	84	−18
9	0.57	+0.61*	0.93	+0.20*	49	+5	996	+20	40	+5	70	+30
10	1.85	+1.97*	4.02	+1.32*	55	+30	2100	+900	40	+20	130	+10
11	1.64	+1.45*	3.76	+0.57*	60	+30	1910	+820	30	+9	140	−14
P	<0.05		<0.01		<0.001		<0.05		<0.001		>0.1	

* Change in cardiac work or oxygen utilization greater than twice the variation observed in duplicate control measurements.

TABLE 2

THE EFFECT OF NICOTINE BASE (20 μG./KG.) ON THE DYNAMICS AND OXYGEN UTILIZATION OF THE HEART DURING MAXIMAL ELECTROCARDIOGRAPHIC ALTERATIONS

Exp. No.	Cardiac work		Oxygen usage		Blood pressure		Cardiac output		Coronary flow		Heart rate	
	Control	Change	Control	Change	Control	Change	Control	Change	Control	Change	Control	Change
	kg.-m./min.		ml./min.		mm. Hg		ml./min.		ml./min.		beats/min.	
1	1.74	+4.81*	5.76	+0.61*	73	+44	1720	+2115	46	+30	100	+10
2	1.61	+0.58*	8.09	+0.52*	85	+14	1391	+237	94	+4	140	−10
3	1.83	+5.76*	8.26	+1.04*	90	+123	1500	+1100	88	+58	130	−20
4	2.81	+0.45*	5.80	+0.95*	60	0	3415	+515	57	+8	170	0
5	1.88	+2.33*	1.31	+3.06*	58	+50	1087	+870	28	+46	140	0
P	0.05		0.05		>0.1		<0.05		<0.05		>0.1	

* Change in cardiac work or oxygen utilization greater than twice the variation observed in duplicate control measurements.

and cardiac oxygen utilization at the onset of the electrocardiographic changes subsequent to the administration of cigarette smoke (1200 to 1500 ml.), nicotine (20 µg./kg. body weight), or epinephrine (5 µg./kg. body weight).

Although cardiac work and cardiac oxygen utilization were found to be increased at the onset of the electrocardiographic alterations subsequent to inhalation of cigarette smoke or administration of the drugs, there was a great variability in the magnitude of the individual responses; in several experiments, the changes noted were insignificant. In several cases, marked electrocardiographic disturbances, ranging from apical premature contractions and multifocal ventricular extrasystoles to ventricular fibrillation, occurred in the absence of significant changes in cardiac work or cardiac oxygen consumption. Conversely, minimal electrocardiographic changes, consisting only of a nodal rhythm, sometimes accompanied marked increases in cardiac work and cardiac oxygen utilization. Coronary blood flow increased in all experiments; however, our experiments did not differentiate between a direct effect of the drug on the coronary arteries and an increased coronary blood flow as a consequence of the increased metabolic demand placed on the myocardium. No relationship was found between the increases in cardiac work and cardiac oxygen utilization, either with respect to each other or with respect to their original levels.

TABLE 3

THE EFFECT OF EPINEPHRINE CHLORIDE (5 µG./KG.) ON THE DYNAMICS AND OXYGEN UTILIZATION OF THE HEART DURING MAXIMAL ELECTROCARDIOGRAPHIC ALTERATIONS

Exp. No.	Cardiac work		Oxygen usage		Blood pressure		Cardiac output		Coronary flow		Heart rate	
	Control	Change	Control	Change	Control	Change	Control	Change	Control	Change	Control	Change
	kg.-m./min.		ml./min.		mm. Hg		ml./min.		ml./min.		beats/min.	
1	1.01	+0.60	0.98	+0.99*	84	+10	1047	+600	30	+14	110	+20
2	0.68	+2.03*	1.11	+3.64*	63	+38	1117	+900	18	+46	150	0
3	1.70	+3.8*	0.59	+2.75*	60	+40	1800	+1000	25	+25	130	0
4	0.55	+1.73*	0.79	+2.83*	52	+60	799	+900	16	+40	120	+40
5	0.80	+1.40*	1.0	+0.96*	68	+10	900	+200	20	+14	110	+40
P	0.05		0.05		0.05		<0.05		<0.01		>0.1	

* Change in cardiac work or oxygen utilization greater than twice the variation observed in duplicate control measurements.

TABLE 1 summarizes the effects of cigarette smoke inhalation on cardiac dynamics and myocardial oxygen utilization at the time of the electrocardiographic alterations. In all experiments there was an increase in cardiac work ($P = <0.05$). In only seven of the experiments, however, could this increase be considered significant as compared to the variation of duplicate control measurements in a given animal. In all experiments in which there was a significant increase in cardiac work, there was a significant increase in cardiac oxygen utilization ($P = <0.01$). In two cases, however, the increase in cardiac oxygen utilization occurred in the absence of a significant change in cardiac work. No relationship could be found between the changes in cardiac work and cardiac oxygen utilization, either with respect to each other or to their original levels. There was no consistent effect noted on heart rate ($P = >0.1$) or coronary arteriovenous difference. Cardiac output ($P = <0.05$), coronary blood flow ($P = <0.001$) and blood pressure increased in all experiments, but there appeared to be considerable variation in the magnitude of the increases.

TABLE 2 summarizes the effects of intravenous injection of nicotine base (20 μg./kg.) on the dynamics and the oxygen utilization of the heart at the onset of the electrocardiographic alterations. As with administration of cigarette smoke, this quantity of nicotine resulted in electrocardiographic alterations ranging from apical premature contractions to multifocal ventricular extrasystoles. Cardiac work increased significantly in all experiments ($P = 0.05$), as did cardiac oxygen consumption ($P = 0.05$). No relationship could be found between the increases in cardiac work and cardiac oxygen consumption, either with respect to each other or to their original levels. There was considerable variability in the changes of blood pressure ($P = >0.1$), cardiac output ($P = <0.05$), coronary blood flow ($P = <0.05$), and heart rate ($P = >0.1$).

TABLE 3 summarizes the effects of epinephrine (5 μg./kg.) on cardiac dynamics and myocardial oxygen utilization. The changes in the measured parameters were less variable following administration of epinephrine than they were following administration of nicotine. With this exception, the results obtained with epinephrine were identical to those of nicotine. Cardiac work and cardiac oxygen consumption increased in all experiments ($P = 0.05$). With the exception of changes in heart rate ($P = >0.1$), blood pressure ($P = 0.05$), cardiac output ($P = <0.05$), and coronary blood flow ($P = <0.01$) increased significantly following administration of epinephrine.

In order to evaluate more completely the changes in cardiac work and cardiac oxygen utilization and to establish a temporal relationship of the electrocardiographic manifestations with these parameters, a continuous record was made of the cardiovascular functions from which the time course of the changes in cardiac work and cardiac oxygen utilization could be calculated. Examples of these records are illustrated in FIGURES 1 and 2 for cigarette smoke, FIGURE 3 for nicotine, and FIGURE 4 for epinephrine.

FIGURES 1 and 2 represent the time course of the changes in cardiac function following administration of cigarette smoke during a 30-sec. period. Although comparison of these charts reveals some temporal variability of response, the general effects are similar in direction and comparable in magnitude. Following administration of cigarette smoke there was a marked increase in cardiac work. Cardiac work remained elevated for a period of approximately 100 sec., after which time it returned to control levels. Immediately upon administration of cigarette smoke in one experiment (FIGURE 1) and after 75 sec. in another (FIGURE 2), there was a marked increase in the oxygen saturation of the coronary venous blood. Arterial blood oxygen saturation did not change during this period. Equating the changes in cardiac arteriovenous difference with the changes in coronary blood flow revealed a precipitous and transient fall in cardiac oxygen utilization. FIGURE 1 illustrates a marked decrease in cardiac oxygen utilization prior to changes in cardiac work. In FIGURE 2, this decrease in cardiac oxygen utilization occurred during a markedly elevated level of cardiac work, presumably at a time when the myocardial metabolic demands were greatly elevated. Both experiments reveal a discrepancy between assumed oxygen requirements and actual oxygen utilization.

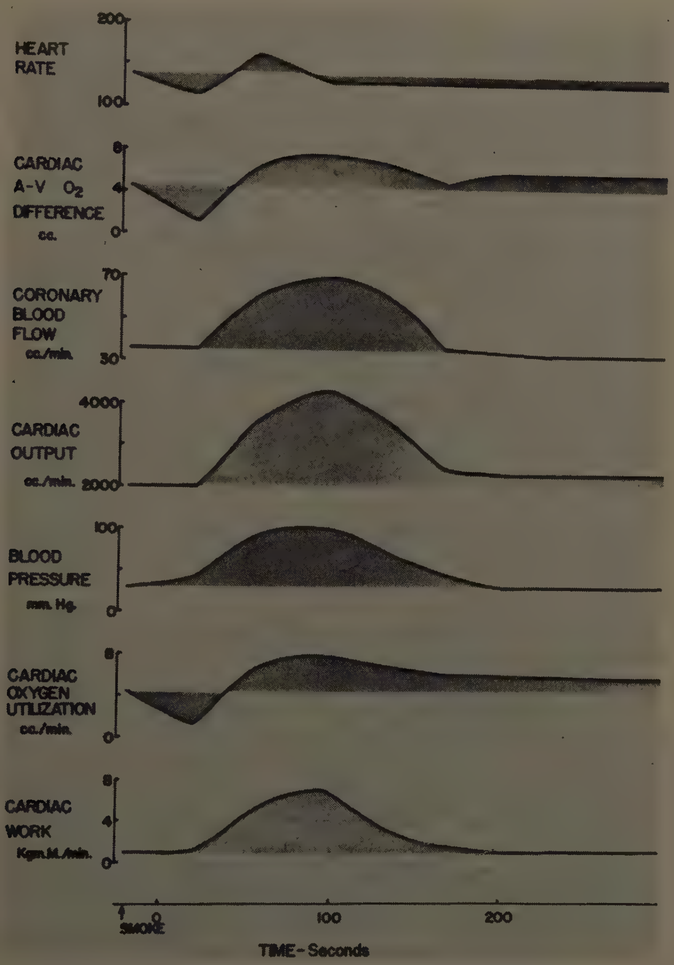

FIGURE 1. The time course of the hemodynamic changes subsequent to administration of 1200 to 1500 ml. of cigarette smoke to an open-chest anesthetized dog. Reproduced with permission from the *Journal of Pharmacology and Experimental Therapeutics* (Kien *et al.*, 1958).

In FIGURE 1 the electrocradiographic alterations began 45 sec. after the cigarette smoke administration, in the absence of changes in cardiac work but in close temporal relationship to the decreased oxygen utilization. In FIGURE 2, the electrocardiographic changes began 125 sec. after administration of cigarette smoke, in the presence of an already elevated level of cardiac work and again, immediately following the decreased cardiac oxygen utilization. In a series of nine experiments it was observed that cigarette smoke inhalation caused a brief but marked reduction in cardiac oxygen utilization. This occurred with great temporal variability during the course of the experiment and with no relationship to the level of cardiac work. In all cases there was a close relationship between the onset of the electrocardiographic alterations and the increased coronary venous blood oxygen saturation. There was no apparent relationship between the degree of decrease in cardiac oxygen utilization and the severity or duration of the electrocardiographic manifestations of cigarette smoke administration.

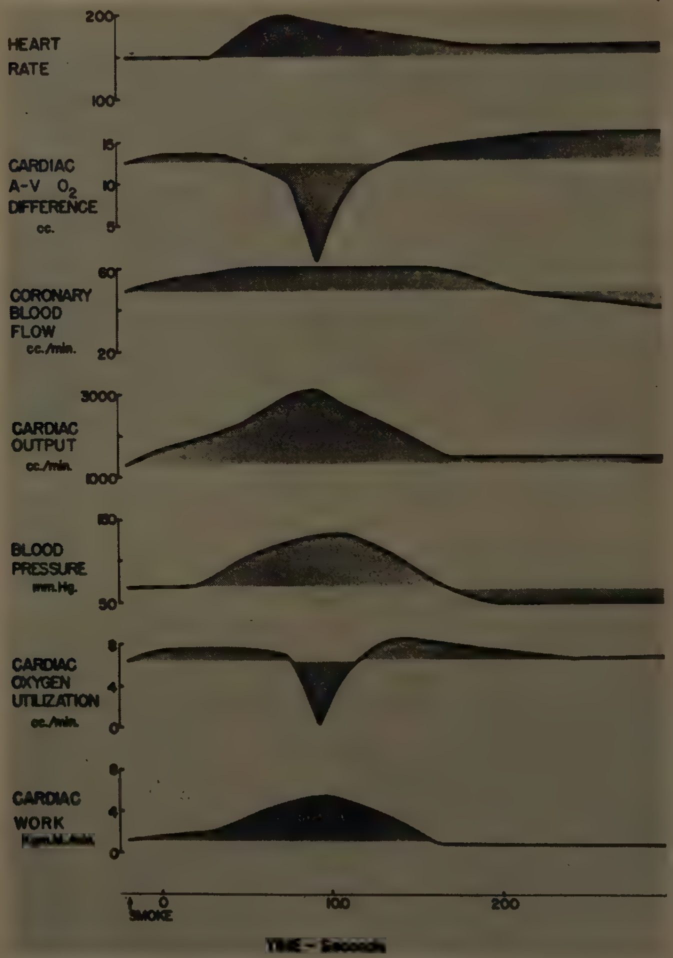

FIGURE 2. The time course of the hemodynamic changes subsequent to administration of 1200 to 1500 ml. of cigarette smoke to an open-chest anesthetized dog.

Administration of nicotine (20 μg./kg. body weight) produced the same cardiovascular response as did cigarette smoke administration (FIGURE 3). In this record, there was a brief but marked fall in cardiac oxygen utilization that occurred approximately 75 sec. after administration of nicotine. Although this fall in cardiac oxygen utilization preceded the marked increase in cardiac work, the discrepancy between apparent oxygen requirement and actual oxygen utilization appeared to be sufficient to initiate severe electrocardiographic alterations. It is probable that the marked rise in cardiac work contributed to the electrocardiographic alterations observed. However, it is of importance to note that the electrocardiographic alterations preceded the increase in cardiac

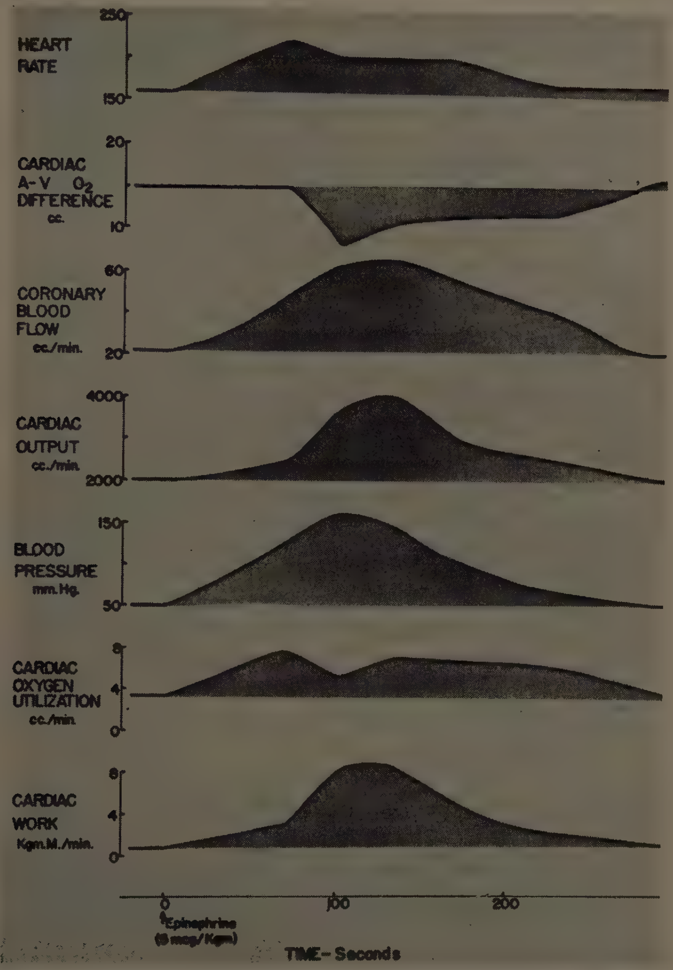

FIGURE 3. The time course of the hemodynamic changes subsequent to administration of 20 μg./kg. body weight of nicotine base. Reproduced with permission from the *Journal of Pharmacology and Experimental Therapeutics* (Kien *et al.*, 1958).

work. This response was observed in a series of six experiments and the graphic representations of these were indistinguishable from those of cigarette smoke administration.

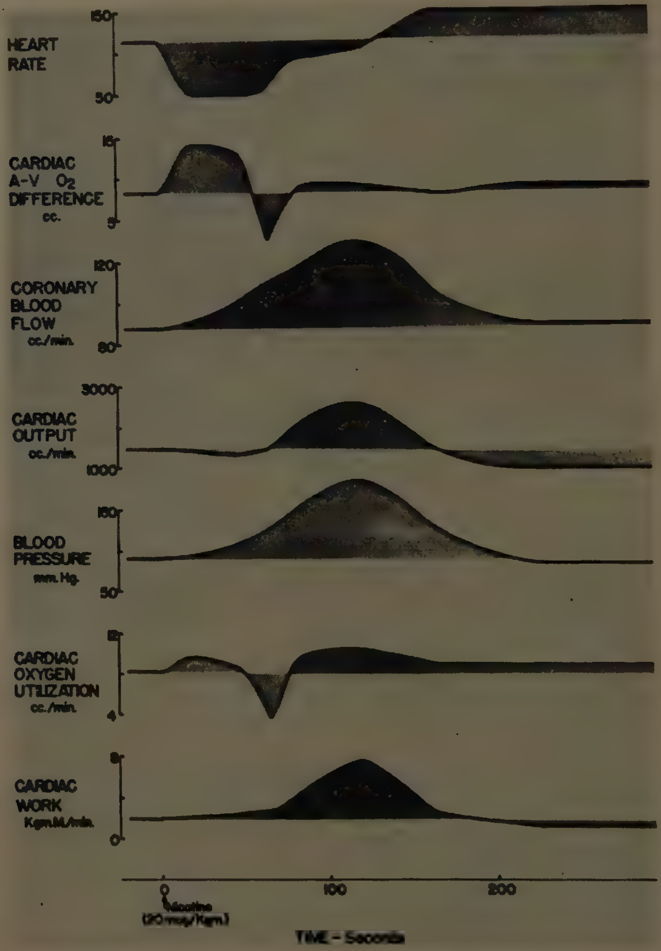

FIGURE 4. The time course of the hemodynamic changes subsequent to administration of 5 μg./kg. body weight of epinephrine chloride.

Except for the absence of the initial bradycardia, administration of epinephrine produced the same cardiovascular responses as those observed following administration of cigarette smoke or nicotine. There was considerably less variability of response following injection of epinephrine than there was following injection of nicotine or inhalation of cigarette smoke. FIGURE 4 represents an average record obtained following injection of five μg./kg. body weight of epinephrine. Of the six experiments in this series, cardiac oxygen utilization fell to below control levels in two cases, to the control level in one, and dipped only slightly in three, as illustrated in FIGURE 4. The decrease in cardiac oxygen consumption following administration of epinephrine occurred consistently during the height of the pressor response and immediately preceded the onset of the electrocardiographic disturbances.

Discussion

Evaluation of the changes in cardiac work at the time of the onset of the electrocardiographic alterations subsequent to cigarette smoke inhalation or administration of nicotine or epinephrine revealed that there was no apparent relationship between these parameters of cardiac activity. It appeared doubtful that an increased level of cardiac work was solely responsible for the electrocardiographic changes on the basis of the observations of only minor electrocardiographic alterations in the presence of greatly increased levels of cardiac work and, conversely, marked electrocardiographic changes with no apparent increase in the level of cardiac work. Coronary blood flow was found to be increased by administration of these pharmacological agents; however, cardiac arteriovenous difference varied considerably and changes in cardiac oxygen utilization did not appear to be correlated with the increased level of cardiac work.

In the series of experiments measuring the time course of the hemodynamic changes following administration of cigarette smoke, nicotine, or epinephrine, there was considerable variability in the magnitude of the responses but, in general, the effects were directionally similar. In the majority of the animals studied, there were marked changes in cardiac output and blood pressure, resulting in greatly elevated levels of cardiac work. Coronary blood flow increased in these experiments. In several experiments in which there was no change in either blood pressure or cardiac output and, hence, no change in cardiac work, there was no change in coronary blood flow. However, it was found that in all animals, regardless of the degree of change in either blood pressure, cardiac output, or coronary blood flow, there was a brief but marked increase in the oxygen saturation of the coronary venous blood. The arterial blood oxygen saturation did not vary significantly from the control values. Equating these changes with the coronary blood flow revealed a diminished cardiac oxygen utilization which occurred, in many experiments, in the presence of an increased workload. It is of interest to note that even in the experiments in which there was no significant change in blood pressure or cardiac output, and hence no change in cardiac work, there nevertheless was a fall in the cardiac oxygen utilization. That this fall in cardiac oxygen utilization was inconsistent with normal cardiac function is suggested by the almost simultaneous occurrence of marked cardiac irregularities.

Bilateral vagotomy prior to administration of cigarette smoke or nicotine failed to alter the extent of the initial decrease in heart rate. Pretreatment with atropine sulfate (1 mg./kg. body weight) completely prevented the bradycardia but had no effect on the subsequent cardiac arrhythmias. The pressor response following administration of cigarette smoke, nicotine, or epinephrine could be prevented entirely by pretreating the animal with piperoxan hydrochloride (Benodain, 2 to 4 mg./kg. body weight); but again, this did not alter the nature or severity of the electrocardiographic alterations. Denervation of the adrenals or bilateral adrenalectomy significantly reduced the pressor response and cardiac arrhythmias elicited by administration of cigarette smoke or the drugs, and pretreatment with tetraethylammoniumchloride (10 mg./kg. body weight) completely abolished all cardiovascular responses (Kien *et al.*, 1958).

On the basis of these data, and the findings of Kiser *et al.* (1956), the cardiac dynamic and oxygen metabolism effects of cigarette smoke or nicotine administration appear to be mediated through the release of epinephrine. Except for the initial bradycardia, the effects of cigarette smoke or nicotine administration could be duplicated by administration of epinephrine. The observed decrease in myocardial oxygen consumption following administration of cigarette smoke or of the drugs, indicates an alteration in the normal cardiac metabolic pattern (Kien and Sherrod, 1960). Such a change, as reflected by the reduction in the cardiac oxygen utilization, occurring in the presence of an alteration of normal cardiac dynamics, could serve as the initiating factor for the observed electrocardiographic alterations. The mechanism for this decreased myocardial oxygen consumption remains to be elucidated.

Summary

The cardiovascular effect of cigarette smoke, nicotine, and epinephrine was studied in the open-chest anesthetized dog. Observations of blood pressure, cardiac output, coronary blood flow, coronary arteriovenous oxygen difference, and the electrocardiogram were made. In addition, cardiac work and cardiac oxygen utilization were calculated.

The maximal cardiovascular effects of cigarette smoke administration were measured in the open-chest anesthetized dog. Extreme variability of response was observed. There was no apparent correlation between the onset of the electrocardiographic effects and the changes in cardiac work. Coronary blood flow increased and appeared to follow the changes in blood pressure and cardiac output. Although the increased cardiac work following administration of cigarette smoke may contribute to the severity of the electrocardiographic alterations, it appears on the basis of the lack of correlation between the electrocardiographic effects and the increased cardiac work, that it might be a secondary factor.

A study of the time course of the hemodynamic changes subsequent to inhalation of cigarette smoke indicated that a brief but marked decrease in the oxygen consumption of the heart occurred in close temporal relationship to the electrocardiographic disturbances. This decreased cardiac oxygen utilization occurred invariably, regardless of the degree or even the existence of changes in cardiac work. It is suggested that this factor is of prime importance in the development of the observed cardiac irregularities.

The effects of cigarette smoke on the dynamics and oxygen utilization of the heart could be duplicated by intravenous administration of nicotine alkaloid or epinephrine.

References

von Ahn, B. 1954. The acute effect of tobacco smoking and nicotine on the electrocardiogram, especially during induced hypoxia: A clinical and experimental investigation. Acta Med. Scand. **292:** 1.

BARGERSON, L. M., D. EHMKE, F. GONLUBOL, A. CASTELLANOS, A. SIEGEL & R. J. BING. 1957. Effect of cigarette smoking on coronary blood flow and myocardial metabolism. Circulation. **15:** 251.

BELLET, S., A. KIRSHBAUM, R. H. MEADE & L. SCHWARTZ. 1941. The effects of tobacco smoke and nicotine on the normal heart and in the presence of myocardial damage produced by coronary ligation. Am. J. Med. Sci. **201:** 40.

DAVIS, F. W., W. R. SCARBOROUGH, R. E. MASON, M. L. SINGEWALD & B. M. BAKER. 1953. The effects of exercise and smoking on the electrocardiograms and ballistocardiograms of normal subjects and patients with coronary artery disease. Am. Heart J. **46:** 529.

DAVIS, F. W., W. R. SCARBOROUGH, R. E. MASON, M. L. SINGEWALD & B. M. BAKER. 1956. The ballistocardiographic cigarette test: further observations. Am. Heart J. **51:** 169.

GREGG, D. E. 1950. Coronary Circulation in Health and Disease. Lea and Febiger. Philadelphia, Pa.

HENDERSON, L. B. 1953. Ballistocardiograms after cigarette smoking in health and in coronary heart disease. Brit. Heart J. **15:** 278.

KIEN, G. A., N. LASKER & T. R. SHERROD. 1957. Action of cigarette smoke on cardiovascular hemodynamics. Federation Proc. **16:** 312.

KIEN, G. A., N. LASKER & T. R. SHERROD. 1958. Action of cigarette smoke on cardiovascular hemodynamics and oxygen utilization in the dog. J. Pharmacol. Exptl. Therap. **124:** 35.

KIEN, G. A. & T. R. SHERROD. 1960. The effect of digoxin on the intermediary metabolism of the heart as measured by glucose-C^{14} utilization in the intact dog. Circulation Research. **8:** 188.

KISER, J. C., W. T. BOOHER & D. T. WATTS. 1956. Blood epinephrine level in dogs following the intravenous administration of nicotine. Arch. Intern. Pharmacodynamie. **105:** 403.

LAUBRY, C., J. WAISER & L. DEGAUDE. 1933. Action experimentale du tabac et de la nicotine sur la debit coronariem. Bull. Acad. Med. **109:** 595.

MANSFIELD, G. & K. HECHT. 1933. Untersuchungen über die wirkung des tabakrauchens auf das herz lungenpräperat von hunden. Arch. Exptl. Pathol. u. Pharmakol. **172:** 362.

RINZER, S. H., D. TRAVELL, D. KARP & D. CHARLESON. 1956. Detection of coronary atherosclerosis in the living rabbit by the eryonovine cigarette test. Am. J. Physiol. **184:** 605.

RUSSEK, H. I., B. L. ZOHMAN & V. J. DORSET. 1955. Effects of tobacco and whiskey on the cardiovascular system. S. African Med. Assoc. **157:** 563.

SCHMITTHENNER, J. E., C. REIGEL & J. H. HAFKENSHIEL. 1956. Effects of increased cardiac work on coronary blood flow and left ventricular metabolism. Nicotine. Federation Proc. **15:** 164.

SHIPLEY, R. E. & C. WILSON. 1951. An improved recording rotameter. Proc. Soc. Exptl. Biol. Med. **78:** 727.

VAN SLYKE, D. D. & J. M. NEILL. 1924. The determination of gases in blood and other solutions by vacuum extraction and manometric measurements. J. Biol. Chem. **61:** 523.

CORONARY BLOOD FLOW AND CARDIAC OXYGEN METABOLISM DURING NICOTINE-INDUCED INCREASES IN LEFT VENTRICULAR WORK*

Ivan E. Forte, A. Jane Williams, Louis Potgieter, J. E. Schmitthenner,†
J. H. Hafkenschiel, Cecilia Riegel
Division of Research, Lankenau Hospital, Philadelphia, Pa.

The authors of the second edition of *The Pharmacological Basis of Therapeutics* state: "There is no proof that the use of tobacco causes arteriosclerosis or results in angina pectoris. However in a rare individual, who already has coronary sclerosis and angina pectoris, over-indulgence in tobacco may occasionally be a factor in precipitating anginal attacks. It is probable that the nicotine-induced increase in blood pressure and in heart rate is the basis for the occasional precipitation of an anginal seizure by smoking."[1] In so far as we are aware the mechanism of anginal seizures has not been precisely defined in terms of the physiological derangements.

In order to obtain more information on this problem, a study of cardiac metabolism was designed, using intravenous infusions of nicotine in intact but anesthetized animals, each dog serving as its own control and returning several times for retesting. Our objective was to simulate in this preparation, under morphine-Dial-urethane-pentobarbital anesthesia (MDUP), a type of investigation that might later be pursued in patients. Specifically, we measured coronary blood flow, using the nitrous oxide desaturation method, cardiac oxygen, glucose, lactate, and pyruvate utilization and left ventricular pressure work in young, healthy dogs with normal coronary arteries. Measurements were made before and during infusions of nicotine that were anticipated to increase heart rate and left ventricular work. The results are compared with data obtained in a study of a newly synthesised pressor substance Hypertensin II peptiden,‡ the same animals and the same experimental design being used in the latter series of experiments under MDUP anesthesia. Hypertensin did increase left ventricular work significantly in this preparation.

We asked this question: When the work load of the left ventricle is increased acutely by an infusion of nicotine or Hypertensin, is the myocardial oxygen extraction coefficient $[(A\text{-}V/A) \times 100]$ increased, unchanged or reduced? Put another way, the question asks whether, during the action of nicotine or Hypertensin, the heart muscle is able to increase its oxygen uptake without reducing the cardiac capillary mean oxygen tension as this might be reflected in cardiac venous blood.

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† Postdoctoral Research Fellow of the National Heart Institute (HF-6498) at Lankenau Hospital, Philadelphia, Pa. (1956–1958).

‡ Made available as Hypertensin-Ciba (BA19990A) by Albert Plummer of Ciba Pharmaceutical Products, Inc., Summit, N. J.

This would result in our animal preparation if coronary blood flow increased more than the cardiac oxygen uptake or demand. Experiments of West[2] suggest that this would occur. In his work nicotine was injected directly into the coronary arteries of dogs lightly anesthetized with pentobarbital sodium, causing increased myocardial vigor and greater coronary sinus blood outflow without inducing any change in heart rate or blood pressure.

Methods

Eight dogs weighing 26 to 46 kilograms were studied. A paired set of observations in each was made at monthly intervals for a period of five to six months. Each animal was anesthetized first with morphine followed by a mixture of pentobarbital and Dial-urethane.

The technique with MDUP anesthesia is as follows. The total morphine dose is 3.0 mg./kg. Two thirds of the initial dose is given subcutaneously 45 min. before the intravenous injection. This consists of equal parts by volume of pentobarbital (65 mg./cc.) and the Dial-urethane mixture, the total being 0.30 cc./kg., that is, 0.15 cc. of pentobarbital and 0.15 cc. of Dial-urethane.* About 1½ hours after the intravenous dose, and after the animal had been operated upon and the catheters placed properly, one sixth of the total dose is administered intramuscularly about 30 min. before the saline infusion for the first measurement of coronary blood flow and cardiac output. Immediately after this study is completed, and approximately 30 min. before the second observation, a comparable dose of morphine is again given intramuscularly. This is usually 20 min. before any drug stimulus is introduced.

An external jugular vein was exposed for catheterization. Two flexible cardiac catheters were positioned under fluoroscopic guidance, one in the coronary sinus and one in the main pulmonary artery, and their positions checked by comparison of blood samples and pressure recordings through a Sanborn electromanometer. A Cournand needle was placed in the femoral artery and connected through a manifold system to a mercury manometer. The animal breathed through a cuffed endotracheal tube connected through a low resistance valve to a spirometer, for collection of expired air and measurement of respiratory minute volume. The expired air was analysed for oxygen and carbon dioxide content by Scholander's method. A leg vein was intubated for infusion of saline during a control period, and of nicotine solution during a drug study period, using a constant rate infusion pump.

Coronary blood flow was measured by the nitrous oxide desaturation method and cardiac output by the direct Fick method.[3,4] Blood samples were withdrawn simultaneously from the femoral artery and coronary sinus (before and midway through the coronary blood flow measurement period to obtain an average sample) for analysis of oxygen and carbon dioxide content; hemoglobin, hematocrit, glucose, lactic acid, and pyruvic acid concentrations.[5] Cardiac rate was determined using limb lead electrocardiographs, usually on lead 2.

Each experiment consisted of a pair of observation periods, 30 to 40 minutes apart, during which measurements of coronary blood flow and cardiac output were made. Infusions were started 5 to 10 minutes before each observation period and continued through it, the infusion time being usually 15 to 25 min. Control saline infusions given during the first observation period were followed by either another control saline infusion or a drug (nicotine or Hypertensin) infusion.

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* Supplied by Ciba Pharmaceutical Products Inc., Summit, N. J.

The two dosage rates of nicotine used, given in a separate series of experiments, were 5 μg./kg./min. and 15 μg./kg./min. The total dose given varied from 3.7 to 4.4 mg. at the lower rate, and from 10.5 to 21.5 mg. at the higher rate. Hypertensin was infused at a rate of 1 μg./kg./min. as previously described.[6] Following the final experiment, each animal was sacrificed, a postmortem examination was made,* the septum divided in half and the left ventricle dissected free and weighed.

All of the data acquired during the paired coronary flow determinations were considered on the basis of the mean change from the first to the second observation. The mean value of the changes from the first control to the second control was compared with the mean value of the changes from the control to the drug observation period. Each drug study included at least four dogs tested with at least four control-control and four control-drug procedures. Statistically, these mean differences were *t*-tested as to the difference of the mean differences. When there was less than 1 per cent chance of coincidence, the change was considered to be pharmacodynamically significant.[7]

Results

This report is based on observations made in 27 experiments on 8 dogs.† In order to obtain this number of good paired experiments some of the tests were excluded for these reasons: altered experimental design, practice experiments, drug response studies (FIGURE 1), failure to keep catheter in coronary sinus confirmed by biochemical data, arterial oxygen saturation below 87 per cent, animal not in steady state—fibrillating or ill (low hemoglobin), failure to intubate coronary sinus, catheter obstructed due to jugular thrombosis, or death of the animal during procedure.

STABILITY OF THE PREPARATION

The results of duplicate determinations completed within approximately 30 minutes on the same dog (MDUP) were essentially the same as the mean changes in the series under pentobarbital anesthesia reported previously, using the same experimental approach for testing the effects of ethyl alcohol,[8] 1-epinephrine, and levarterenol.[9] These findings confirm Foltz and co-workers.[10] All of the animals considered here were tested under MDUP anesthesia as to the effects of nicotine and Hypertensin.

REPEATED OBSERVATIONS OF CORONARY BLOOD FLOW AND CARDIAC OXYGEN CONSUMPTION IN THE SAME ANIMALS

In order to compare our experimental approach with that reported by Foltz,[10] we made repeated observations on a smaller number of animals. The mean value of coronary blood flow was 91, with S.D. ± 31, and cardiac oxygen consumption was 11.6, with S.D. ± 4.6, in 25 experiments on 8 dogs weighing from 26 to 48 kg. These observations showed us that this variation is essentially the same as that reported by Foltz.

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* Courtesy of James F. Welsh, Department of Pathology, Lankenau Hospital.

† Including 25 experiments with coronary flow measurements.

Comparison of Two or More Observations on the Same Dog

The mean values obtained in the first run were compared with the second run mean and the standard deviation of the individual differences was calculated. Of interest in relation to "the multiplicity of possible errors in the nitrous oxide procedure"[11] are the 37 comparisons using 8 dogs while under MDUP anesthesia. The standard deviations of the coronary blood flow (S.D. ± 27), and the cardiac oxygen consumption (S.D. ± 4.4), are essentially the same as in experiments described above.

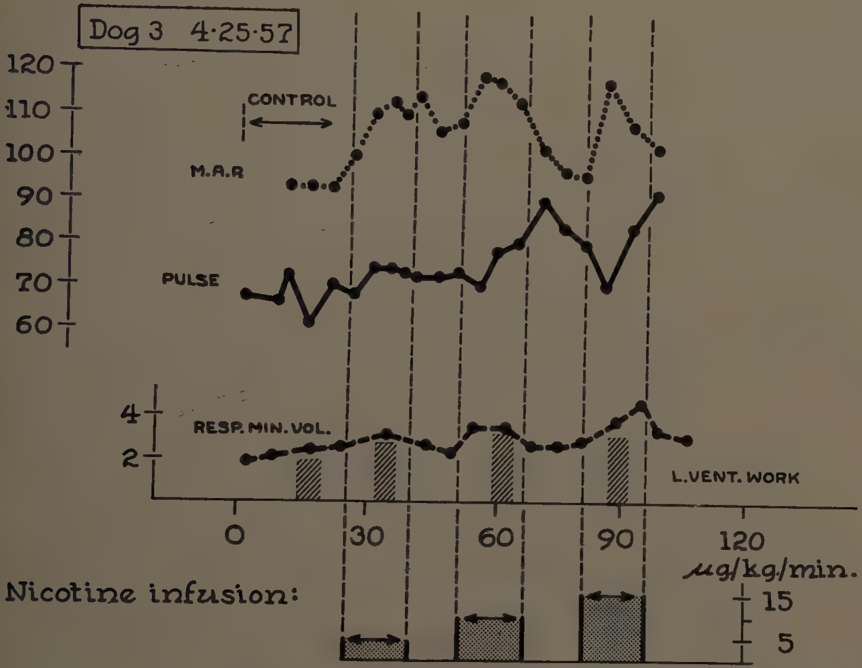

FIGURE 1. Nicotine infusion. This figure indicates the values of the different parameters during a control period and the changes, with time in minutes, as three different concentrations of nicotine were infused into a dog. M.A.P. is the mean arterial blood pressure in millimeters of mercury; pulse the heart rate per minute; resp. min. vol. is the respiratory minute volume in liters per minute; and L. vent. work is the work of the left ventricle in kilogram meters per minute. This graph shows that the induction of a "steady" change in mean arterial pressure and heart rate is improbable in the dosage range 5 to 15 µg./kg./min. See text.

The conclusion of this analysis is that the method would appear to have validity in measuring coronary blood flow changes, not only from observation period number 1 in a given day to observation period number 2 some 30 to 40 minutes later, but also from period number 1 today to period number 1 on the same dog under the same conditions, at approximately four-week intervals. We believe we have confirmed Gregg's conclusion that "the method is believed to indicate, semiquantitatively, coronary flow per minute per 100 gm. of left heart."[11,12]

CHANGES INDUCED BY NICOTINE AND HYPERTENSIN

Dosage of Nicotine Used for Intravenous Infusion

One of the problems met at the outset was the difficulty in obtaining a preparation of nicotine the dose response of which could be precisely quantitated. It was not until Julius Comroe and the Scientific Advisory Board of the Tobacco Industry Research Committee, New York, N. Y., made available to us standardized ampoules (2.0 cc. with 2 mg./cc.) that we were able to get consistent results using the constant rate infusion method. The most reproducible results hemodynamically were at an infusion rate of 5 μg./kg./min. A dose response experiment is shown in FIGURE 1.

Although there are effects on heart rate, arterial pressure and respiration with an infusion of 5 μg./kg./min. for 20 to 30 min., there were no statistically significant ($P < 0.01$) differences in the mean changes in the experiments conducted using the smaller dosage. In this group of experiments the total dose of nicotine infused was 4.0 mg. This dose was selected initially because it was considered to be the equivalent of smoking one standard cigarette.[13]

Using a total dose of nicotine over 20 mg. in dose response experiments, we observed that this amount induced cardiac arrhythmia, fall of blood pressure, and respiratory failure. Accordingly, a series of experiments were conducted using only 15 μg./kg./min., or a total dose of about 12.0 mg. (possibly the equivalent of smoking 3 standard cigarettes over a half-hour period). The mean changes with the larger nicotine dose are compared in TABLE 1 with the effects of the smaller dose, the mean change in the double control series (anesthesia alone), and the mean changes during the Hypertensin infusion.

Intravenous Infusions of Nicotine 15 μg./kg./min. for 20 to 30 min.

Hemodynamic changes. In FIGURE 2 is shown the continuous relationship between the average changes in mean arterial pressure and heart rate during all infusions from minute to minute. The blood pressure rose in every instance, but to varying degrees, and would then fall off, with heart rate increasing. In several experiments pressure fell below the preinfusion level, while the nicotine was still being infused.

Heart rate initially increased. This dipped as the pressure increased, then became faster to a peak point, after which the rate became slower, usually returning close to the base-line level within 10 minutes after the infusion was stopped. These varying levels are to be contrasted with the steady state patterns seen in the same animals when saline was infused. The dotted lines in FIGURE 2 represent the changes occurring in the same animals when studies were done at other times, usually at four week intervals, using saline in the second period, and are labeled as control-control (or anesthesia alone) experiments.

TABLE 1

MEAN VALUES OF CHANGE IN HEMODYNAMIC AND CARDIAC OXYGEN METABOLIC OBSERVATIONS ON THE SAME DOGS, THE SECOND OBSERVATION MADE AFTER 20 MIN. OF INFUSION OF NICOTINE*

	First run	Nicotine		Anesthesia alone	Hypertensin II Peptiden 1 μg./kg./min.
		Change with 15 μg.	5 μg.		
Coronary blood flow (ml./100 gm./min.)	78	53	4	7	99
Cardiac O_2 consumption (ml./100 gm./min.)	9.7	8.7	0.7	1.6	12.6
Left ventricular work (kg.-m./min.)	5.1	2.3	0.4	−0.1	2.2†
Cardiac output (ml./min.)	3370	1080	100	−250	−400
MABP (mm. Hg)	112	11	4	7	66†
Cardiac rate (per min.)	66	45†	−2	10	11
Coronary resistance (P. ÷ C.F.)	1.51	−0.51	−0.03	0.09	−0.27
Arterial Hb. (gm./100 ml.)	14.3	1.2	0.2	0.1	1.7†
Coronary A_{O_2}-V_{O_2} (Vol. %)	12.4	1.6	0.4	0.8	0.8
Myocardial oxygen coefficient extraction (%)	72	−1	−2	3	0
Coronary sinus venous O_2 saturation (%)	26	2	3	−3	0
Coronary sinus venous O_2 tension (mm. Hg)	20	1	1	−2	1

* Five μg./kg./min. and 15 μg./kg./min.

† Denotes pharmacodynamically significant changes with $P < 0.01$.

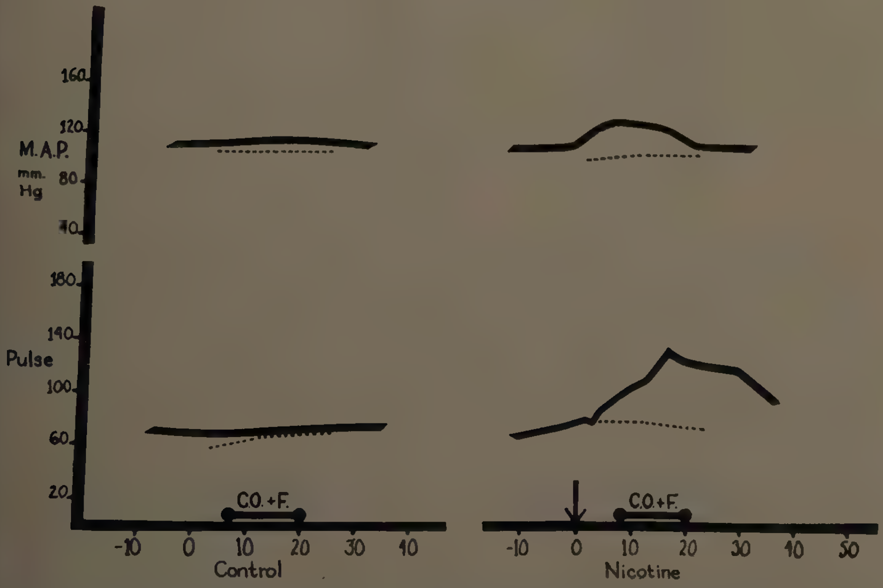

FIGURE 2. This diagram shows how each experiment consisted of two observation periods, the abscissa giving the time in minutes. The dotted lines represent the changes in the pulse and mean arterial blood pressure (M.A.P.) when saline was infused in the control-control experiments. The continuous dark lines represent the average of changes occuring in these parameters from the control periods on the left to nicotine infusions on the right, the upper line being mean arterial blood pressure in millimeters of mercury and the lower line the pulse in beats per minute. See text.

Our calculations using mean arterial pressure for left ventricular work as shown in TABLE 1 are based on the *average* mean arterial pressure during the nicotine infusion period and do not reflect the extent of variation observed.

Although mean heart rate was increased (66 to 115) there was no significant mean increase in pressure or left ventricular work. Both coronary blood flow and cardiac output were measured during a changing circulatory (and perhaps metabolic) state, rather than during a hemodynamically steady state as required by both the nitrous oxide method of measuring coronary blood flow and the Fick technique for cardiac output. The changes in coronary blood flow were not consistent, although in individual experiments such as that of March 30, 1959, on Dog B (FIGURE 3), the alterations were much as for the entire series average. In this experiment the flow increased from 64 to 105 cc. with cardiac oxygen uptake almost doubled (from 7.8 to 14.1 cc.), heart rate increased from 51 to 84, cardiac output from 2.1 to 3.8, and left ventricular work rose from 3.4 to 7.3 kg.-m./min.

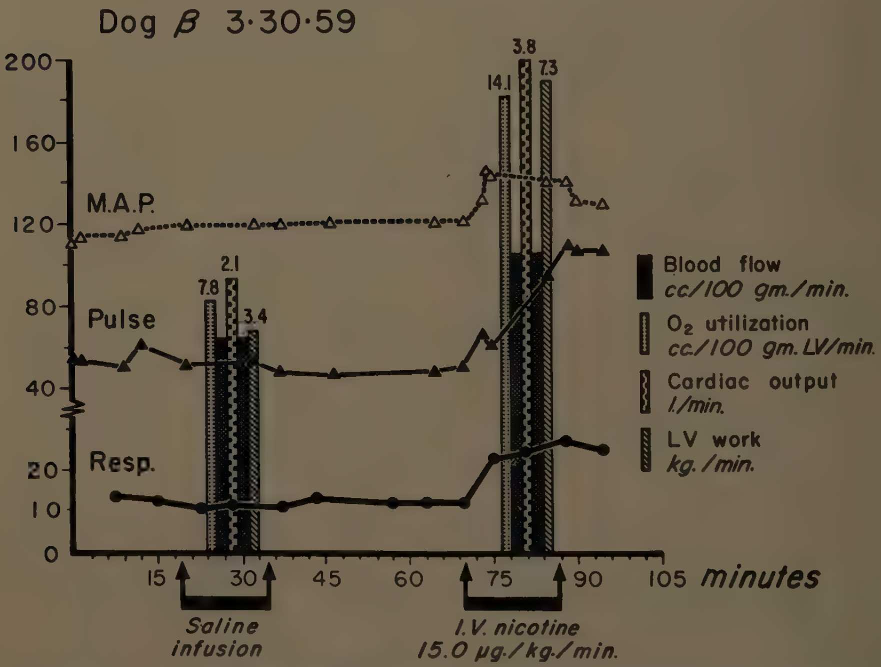

FIGURE 3. Dog B. This graph is representative of paired control-nicotine intravenous infusion experiments. The only significant change for the group as a whole was the increase in heart rate during the nicotine infusion. The saline infusion in minutes serves as the control. The amount of intravenous (I.V.) infusion of nicotine for 15 min. was 15 μg./kg./min. M.A.P. on the broken line graph is for the mean arterial blood pressure in millimeters of mercury. Pulse is the heart rate per minute and Resp. the respiratory rate per minute. For the bar graphs the key is in the right hand corner. Starting at the top, the coronary blood flow is in cubic centimeters per 100 grams left ventricle per minute; cardiac oxygen utilization, as cubic centimeters per 100 gm. left ventricle per minute; cardiac output as liters per minute and left ventricular work in kilogram-meters per minute.

Coronary oxygen arteriovenous difference was not significantly increased and the myocardial oxygen extraction coefficient, 72 per cent initially, was unchanged. Coronary sinus venous oxygen tension remained at 20 mm. Hg (TABLE 1).

Metabolic parameters. The mean changes are shown in TABLE 2. The only significant change in cardiac fuel was the increased cardiac lactate utilization during the Hypertensin infusion. We discern no evidence that nicotine induces a shift to anerobic metabolism.

TABLE 2

CARDIAC CARBOHYDRATE METABOLITE CHANGES DURING THE INFUSION OF NICOTINE (15 μg./kg./min.) and Hypertensin II Peptiden (1 μg./kg./min.)

	Pyruvate				Lactate				Glucose			
	Art. mg. %	A-V mg. %	C. Ext. %	L.V. util.	Art. mg. %	A-V mg. %	C. Ext. %	L.V. util.	Art. mg. %	A-V mg. %	C. Ext. %	L.V. util.
(A) 15 μg. Nicotine												
Before	1.11	0.53	38.2	0.48	10.6	3.9	31.6	3.4	74	3	3	2
During	1.16	0.50	33.4	0.68	13.8	3.3	20.9	4.4	92	2	2	3
Change	0.05	−0.03	−4.8	0.20	3.2	−0.6	−10.7	1.0	18	−1	−1	1
(B) 1 μg. Hypertensin												
Before	0.77	0.30	34.5	0.36	7.7	2.4	28.7	2.5	79	6	7	4
During	1.14	0.53	45.7	0.82	15.6	6.5	40.2	10.7	107	10	9	19
Change	0.37	0.23	11.2	0.46	7.9*	4.1*	11.5	8.2*	28*	4	2	15
(C) Double Controls†												
Before	0.79	0.29	32.7	0.21	7.7	2.3	29.7	1.9	69	2	3	2
During	0.92	0.39	36.1	0.28	8.0	2.3	26.1	1.8	75	5	7	5
Change	0.13	0.10	3.4	0.07	0.3	0	−3.6	−0.1	6	3	4	3

Abbreviations: A-V, coronary arteriovenous difference; C. Ext., coefficient of extraction (A-V/A); L.V. util., left ventricular utilization in mg./100 gm./min.

* Denotes pharmacodynamically significant changes with $P < 0.01$.

† Same animals that were tested with high dose of nicotine.

The higher nicotine dosage did stimulate respiration. The greater ventilation led to an increased arterial O_2 content. The coronary sinus oxygen content showed a mean increase from 4.9 to 5.9 ml./100 ml., the arterial change being from 17.3 to 19.8 ml., but the cardiac oxygen extraction did not change. However, these changes, as well as the change in arterial glucose with nicotine, were not significant at $P < 0.01$.

Discussion

The original purpose of these experiments, that is, to increase left ventricular work by an infusion of nicotine, was not accomplished. The reasons for this, which are probably primarily related to the varying arterial pressure response and inconsistent changes in cardiac output, have been mentioned (FIGURE 2). The Hypertensin experiments did show that this latter substance, presumably having a primary and probably an exclusively vasoconstrictor action, does increase both mean arterial pressure and left ventricular work in our animal preparation.

TABLE 3
LEFT VENTRICULAR WORK AND CARDIAC OXYGEN UTILIZATION
Percent Change of Observations Repeated 30 Min. Later

	Pentobarbital			Morphine-Dial-urethane-pentobarbital		
	Anes. alone	1-Epin.	Levarterenol	Anes. alone	Nicotine	Hypertensin II Peptiden
Coronary flow						
% Change	10	34	−1	−3	67	94
% Coef. var.	22	52	47	26	55	127
Cor. A-V O_2						
% Change	4	−40*	−6	6	13	6
% Coef. var.	15	21	17	7	9	20
Card. O_2 cons.						
% Change	13	−37	−11	0	90	89
% Coef. var.	19	32	27	25	68	87
Card. output						
% Change	−12	43	−6	−6	32	−11
% Coef. var.	18	73	65	22	27	14
MABP						
% Change	−2	25	10	6	10	54*
% Coef. var.	9	15	11	10	12	17
L. vent. work						
% Change	−13	90	2	−2	45	37*
% Coef. var.	19	80	68	24	35	14

Abbreviations: Cor., coronary; Card., cardiac; A-V, arteriovenous difference; Cons., consumption; MABP, mean arterial blood pressure; L. vent., left ventricular.

* Denotes a statistically significant difference when compared with the "anesthesia alone" changes ($P < 0.01$).

Anesthesia alone represents the control-to-control observations.

1-Epinephrine infused at rate of 3 μg./kg./min.

Levarterenol infused at rate of 3 μg./kg./min.

Nicotine infused at rate of 15 μg./kg./min.

Hypertensin II Peptiden infused at rate of 1 μg./kg./min.

% Change from first to second period of observation.

% Coefficient of variation, the ratio of the standard deviation of the individual difference to the initial value of the parameter.

Nicotine in the higher dosage experiments does increase heart rate. However within the requirements of our experimental design, we did not record a statistically significant increase in cardiac oxygen utilization. Because of the wide coefficient of variation in coronary blood flow values in all of our experiments, including those under pentobarbital anesthesia (shown in TABLE 3 as percent coefficient of variation), we suspect that this failure to record a statistically significant increase in cardiac oxygen consumption is not "indicative of rather wide biological variation between animals,"[10] but rather a limitation of the nitrous oxide method, when applied under the conditions of these experiments, that is, a changing state from moment to moment.[12]

Shown also in TABLE 3 are the changes in cardiac arteriovenous oxygen differences during the action of all drugs tested in our laboratory. Nicotine stimulated respiratory activity primarily by increasing the depth of breathing so that in this group of animals the arterial oxygen content was increased, but with the coronary arteriovenous difference increasing only 13 per cent, the coronary sinus oxygen content and oxygen tension were unchanged. This suggests that coronary blood flow in most nicotine experiments increased enough or more than enough to meet the oxygen metabolic needs. This finding is similar to that reported by West, using a similar dog preparation and anesthesia, as a result of the action of 1-epinephrine or levarterenol infusion.[14] This implies that the myocardial oxygen extraction coefficient does not have to be increased during the increased contractile vigor induced by nicotine[2] because the coronary flow adjustment is adequate. Säyen and his co-workers have reviewed the pertinent literature on responses of myocardial oxygen, coronary venous oxygen and coronary flow to levarternol and epinephrine.[15]

The results of these nicotine infusion studies in the morphine-Dial-urethane-pentobarbitalized dog would appear to support the concept that coronary blood flow adjusts itself to meet the demands of the myocardium for oxygen, rather than the coronary arteriolar bed being effected per se by drugs.[16,17] The precise adjustment of the coronary flow seen here in the presence of the increase in heart rate upholds the thesis of Shipley and Gregg that the primary determinants regulating coronary flow are the vigor of cardiac contraction and the increase in cardiac oxygen metabolism.[18] Although the cardiac oxygen utilization was not increased to a statistically significant degree, the oxygen demand must have been at a higher level with the greater heart rate. Inasmuch as our studies were not designed to elucidate mechanisms involved, we can surmise only that this adjustment is by central nervous system pathways, possibly through the sympathetic division. Eckstein and his co-workers were unable to demonstrate humoral vasoactive or cardiotropic substances in the hypoxic and overperfused myocardium in their experiments.[19]

These observations have been made in dogs with a normal coronary vasculature. In these test objects, adjustments of vascular tone are apparently made easily. The reactivity of the abnormal or sclerotic arteries is probably entirely different. If the vessels are fixed in caliber their tonus cannot change, so that the fine adjustment is removed and only the coarse adjustment (level of arterial pressure) remains. Also unknown are the factors both in healthy and diseased myocardial cells that allow for a more efficient extraction of metabolites or conversions of chemical to mechanical energy when the central nervous system is stimulated appropriately. If under conditions of a fixed vessel caliber, a change in contractile force requires a greater oxygen supply, the coronary flow may be less than adequate for the altered metabolic needs of the myocardium and relative coronary insufficiency may ensue.

Thus our experiments suggest that with a healthy coronary arterial tree the action of nicotine in increasing heart rate does not adversely affect the myocardium. This lack of untoward effect on the heart and the pronounced effect on depth of respiration suggests a possible clinical application. Would nicotine used under the conditions herein described have any usefulness as an adjunctive stimulus in the management of those patients who have tried to destroy themselves by overdosages of barbiturates, when they are not responding to the conventional measures?

From the experiments of West[2] showing the complex myocardial effects of intracoronary arterial injections of nicotine and the complex action of nicotine in a simpler preparation,[20] it is understandable why we were not able to induce a steady state as to blood pressure level and heart rate during the infusion similar to the relatively steady state observed during the infusion of Hypertensin. We observed no significant changes in the mean response with the 5 μg. nicotine infusion, but some mean changes in the small group of animals studied during the 15 μg. infusion. Our results, indicating that coronary blood flow was not reduced and that the cardiac oxygen utilization was met without reducing the level of oxygen tension in myocardial venous blood, appear to confirm the findings of West *et al.*, that nicotine in "healthy coronary arteries does not cause constriction."[2] A similar finding, using the nitrous oxide method of measuring coronary blood flow during cigarette smoking in normal volunteers, has been reported by Bargeron.[21]

The following hypothesis is suggested to explain how tobacco smoking in a patient with coronary sclerosis may be a factor in precipitating an occasional anginal attack. Such a patient with diseased coronary arteries is unable to increase coronary blood flow (cardiac oxygen delivery) in order to meet the greater myocardial oxygen demands. These are induced by the greater contractile force and the increased heart rate associated with the action of nicotine inhaled in excessive smoking. When the cardiac venous oxygen reserve is encroached upon, either the buildup of waste products of metabolism and hypoxia per se, myocardial ganglionic liberation of catecholamines, or reflexes activating the central nervous system through the sympathoadrenal axis of the autonomic division produce symptoms that physicians recognize as pain of cardiac origin and call angina pectoris.

Further studies of this type are desirable in patients with coronary artery disease to test this hypothesis. Our present position is that a simpler method of quantitating coronary blood flow in the unanesthetized patient must be devised before we would be willing to undertake this investigation in coronary artery disease patients.[12,22]

References

1. Goodman, L. S. & A. Gilman. 1955. The Pharmacological Basis of Therapeutics. : 625. Macmillan. New York, N. Y.
2. West, J. W., S. V. Guzman & S. Bellet. 1958. Cardiac effects of intracoronary arterial injections of nicotine. Circulation Research. **6:** 389.
3. Goodale, W. T. & D. B. Hackel. 1953. Measurement of coronary blood flow in dogs and man from rate of myocardial nitrous oxide desaturation. Circulation Research. **1:** 502.
4. Eckenhoff, J. E., J. H. Hafkenschiel, M. H. Harmel, W. T. Goodale, M. Lubin, R. J. Bing & S. S. Kety. 1948. Measurement of coronary blood flow by the nitrous oxide method. Am. J. Physiol. **152:** 356.
5. Goodale, W. T., M. Lubin, J. E. Eckenhoff, J. H. Hafkenschiel & W. G. Banfield, Jr. 1948. Coronary sinus catheterization for studying coronary blood flow and myocardial metabolism. Am. J. Physiol. **152:** 340.
6. Potgieter, L., J. E. Schmitthenner, I. Forte, A. J. Williams & J. H. Hafkenschiel. 1959. Hypertensin-II-peptiden: Effect of infusions on coronary flow and cardiopulmonary mechanisms. Federation Proc. **18:** 481.

7. Albritton, E. C. 1949. Experimental Design and Judgement of Evidence. Edwards. Ann Arbor, Mich.
8. Schmitthenner, J. E., I. Forte, A. J. Williams, J. H. Hafkenschiel & C. Riegel. 1958. Does alcohol increase coronary blood flow and cardiac work? Circulation. **18:** 778.
9. Schmitthenner, J. E., I. Forte, J. H. Hafkenschiel & C. Riegel. 1958. Left ventricular cation exchange: Effects of epinephrine and norepinephrine. Federation Proc. **17:** 143.
10. Foltz, E. L., R. G. Page, W. F. Sheldon, S. K. Wong, W. J. Tuddenham & A. J. Weiss. 1950. Factors in variation and regulation of coronary blood flow in intact anesthetized dogs. Am. J. Physiol. **162:** 521.
11. Gregg, D. E., F. H. Longino, P. A. Green & L. A. Czerwonka. 1951. A comparison of coronary flow determined by the nitrous oxide method and by a direct method using the rotameter. Circulation. **3:** 89.
12. Forte, I., A. J. Williams, J. E. Schmitthenner, H. Neal & J. H. Hafkenschiel. 1960. Practical limitations of the nitrous oxide method for the determination of regional blood flow. Clin. Research. **8:** 182.
13. Roth, G. M. 1951. Tobacco and the Cardiovascular System. American Lecture Series. Thomas. Springfield, Ill.
14. West, J. W., A. Mercker, H. E. Lessner & E. Mezger. 1954. Effect of continuous intravenous infusion of epinephrine and norepinephrine on coronary sinus blood oxygen saturation. Federation Proc. **13:** 163.
15. Säyen, J. J., A. H. Katcher, W. F. Sheldon & C. M. Gilbert. 1960. The effect of levarterenol on polarographic myocardial oxygen, the epicardial electrocardiogram and contraction. Circulation Research. **8:** 109.
16. Eckenhoff, J. E., J. H. Hafkenschiel, C. M. Landmesser & M. Harmel. 1947. Cardiac oxygen metabolism and control of coronary circulation. Am. J. Physiol. **149:** 634.
17. Eckenhoff, J. E. & J. H. Hafkenschiel. 1947. The effect of nikethamide on coronary blood flow and cardiac oxygen metabolism. J. Pharmacol. Exptl. Therap. **91:** 362.
18. Shipley, R. E. & D. E. Gregg. 1945. The cardiac response to stimulation of the stellate ganglia and cardiac nerves. Am. J. Physiol. **143:** 396.
19. Jelliffe, R. W., C. R. Wolf, R. M. Berne & R. W. Eckstein. 1957. Absence of vasoactive and cardiotropic substances in coronary sinus blood of dogs. Circulation Research. **5:** 382.
20. Trendelenburg, U. 1959. Non-nicotinic ganglion-stimulating substances. Federation Proc. **18:** 1001.
21. Bargeron, L. M., Jr., D. Ehmke, F. Gonlubol, A. Castellanos, A. Siegel & R. J. Bing. 1957. Effect of cigarette smoking on coronary blood flow and myocardial metabolism. Circulation. **15:** 251.
22. Forte, I., A. J. Williams, J. E. Schmitthenner, H. Neal, H. Woske, R. Richards & J. H. Hafkenschiel. 1960. Coronary blood flow using radioactive iodine compared with nitrous oxide. Federation Proc. **19:** 90.

EFFECT OF CIGARETTE SMOKING ON CORONARY CIRCULATION AND CARDIAC WORK IN PATIENTS WITH ARTERIOSCLEROTIC CORONARY DISEASE*

Timothy J. Regan, Harper K. Hellems, Richard J. Bing
Wayne State University College of Medicine, Detroit, Mich.

In a preceding publication from this laboratory, the effect of cigarette smoking on coronary blood flow and myocardial metabolism was investigated by means of coronary sinus catheterization.[1] It was found that the smoking of one cigarette significantly increased the coronary blood flow and heart rate and produced a fall in coronary vascular resistance and extraction of oxygen and glucose. Although these studies suggested that in healthy individuals smoking of cigarettes does not result in coronary vasoconstriction, the possibility existed that smoking in patients with arteriosclerotic heart disease diminishes the coronary blood flow. It has been shown, for example, by a large number of workers that smoking produces T-wave depression and sagging of the ST segment in patients with coronary heart disease.[2] It has also been demonstrated that in these patients smoking results in definitive changes in the ballistocardiogram.[3] These changes have been interpreted as resulting from a deterioration of cardiac function during smoking, possibly as a result of diminished coronary blood flow. Anginal pain during smoking has been observed by a series of clinicians. Although angina is rarely if ever produced by smoking, it has been observed that the symptoms may disappear on omitting the use of tobacco.[4] The present investigation was undertaken to study the effect of cigarette smoking on coronary blood flow, cardiac output, peripheral blood pressure, and pulse rate in individuals with arteriosclerotic heart disease.

Procedures

Seven male individuals were studied. All had proved electrocardiographic and clinical evidence of myocardial infarction that had occurred from seven months to two years preceding the test. All patients suffered from angina pectoris during exertion, and the pain was relieved by nitroglycerin. Only three of the patients (W.T., S.K., E.Z., TABLE 1) had positive Master's tests at the time of catheterization. None of the patients were in clinical congestive failure, and the heart was not enlarged by X ray. In all subjects peripheral venous and right atrial pressures were found to be normal on cardiac catheterization.

The patients were studied in the following manner. The coronary sinus was catheterized in the usual manner, using a No. 7 catheter. Following this, a second catheter, No. 6, was introduced through the same vein and the tip was placed into the right atrium. An indwelling needle was inserted into a brachial artery. The expired air was then collected in a Tissot spirometer for analysis of expired air and computation of O_2 consumption. Simultaneously,

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right atrial and brachial artery samples were taken after the coronary flow samples for determination of the cardiac output by means of the Fick principle, and brachial artery and right atrial pressures were recorded. Coronary blood flow was measured by the nitrous oxide method, using the desaturation method previously described. The patient then smoked two cigarettes over a 25-min. period, inhaling the smoke and taking a normal puff on the cigarette about once a minute. Blood pressure and heart rate were recorded during this period. The patient then inhaled the N_2O mixture for 15 min. Immediately afterwards he smoked a third cigarette, during which the coronary blood flow was determined. Blood pressure and pulse rate were repeatedly measured and the cardiac output was taken at the conclusion of the determination of coronary flow. Oxygen content of blood was determined on the Van Slyke apparatus with the method of Van Slyke and O'Neill; nitrous oxide was measured accord-

TABLE 1

CARDIOVASCULAR EFFECTS OF CIGARETTE SMOKING IN PATIENTS WITH CORONARY ARTERY DISEASE

Case, age, body surface area	Coronary blood flow (ml./100 gm./min.)	O_2 arteriovenous difference	O_2 consumption (ml./100 gm./min.)	Total body O_2 consumption (ml./min.)	Cardiac index (l./min.)	Pulse rate	Mean arterial pressure (mm. Hg)	Left ventricular work (kg./m./min.)	Ratio Left ventricular work / O_2 consumption
J.F., 46, 2.07	82*	13.88	11.38	272	2.29	99	85	5.77	0.51
	71†	13.49	9.94	294	2.50	110	80	5.92	0.60
S.K., 58, 1.82	60	11.23	6.73	150	1.78	73	83	3.86	0.57
	61	11.59	7.04	208	2.10	86	100	5.48	0.78
W.T., 53, 2.01	90	10.70	9.58	286	3.18	82	120	11.01	1.14
	71	9.80	6.95	261	3.73	100	135	14.49	2.04
A.G., 46, 1.43	80	11.53	9.18	199	3.57	92	80	5.84	0.64
	77	11.52	8.91	184	4.32	130	100	8.84	0.99
E.Z., 48, 1.88	73	10.48	7.60	212	2.56	70	87	7.40	0.97
	74	12.86	9.48	222	5.16	82	95	13.39	1.14
E.P., 43, 1.94	51	13.00	6.64	197	1.08	57	115	3.46	0.52
	72	12.39	8.92	239	2.18	59	130	7.87	0.88
L.S., 39, 2.24	67	13.98	9.34	311	2.49	88	92	7.35	0.79
	67	13.86	9.23	290	2.17	95	127	8.35	0.90
Mean values	72	12.11	8.63	232	2.42	80	95	6.38	0.73
	70	12.22	8.63	243	3.17	95	109	9.16	1.05
P values	‡	‡	‡	‡	>0.05	<0.02	>0.05	<0.05	<0.01

* Control values.
† During cigarette smoking.
‡ Not statistically significant.

ing to the method of Kety. Standard methods of calculating external heart work and efficiency were employed.[5,6]

Results and Discussion

TABLE 1 illustrates that smoking resulted in an increased heart rate in each patient. In six of seven patients, arterial blood pressure rose an average of about 10 per cent of the control value, as did the cardiac output. As a result of these changes, the left ventricular external work rose from an average of 6.38 to 9.16 kg./m./min.

In three of the patients the coronary blood flow did not change, while in three there was a slight fall. With one exception none showed the increase in coronary flow previously observed in normal individuals during smoking. The coronary arterial-venous oxygen difference did not change. The myocardial oxygen consumption increased very slightly in three of the seven patients. Since the work of the heart increased proportionately more than the oxygen consumption, the myocardial efficiency increased an average of 15 per cent.

Data reported in this paper illustrate that smoking in patients with coronary heart disease results in no appreciable changes in coronary blood flow and myocardial oxygen consumption. However the heart rate, blood pressure, cardiac output, and work of the left ventricle increase. The observation that coronary blood flow fails to increase in the patients of this series is in contrast to previously published findings on normal individuals, who demonstrated a significant increase in coronary blood flow with presence of diminished left ventricle work.[1] This finding suggests that the electrocardiogram and ballistocardiogram changes observed during smoking are not due to a diminution in the coronary blood flow; instead, they appear to be the result of a relatively deficient oxygen supply to the myocardium in the presence of increased oxygen demands, conditioned by increase in cardiac work. This is illustrated by the finding that the ratio of work over oxygen consumption increased in every single patient (TABLE 1). It has been shown that nitroglycerin fails to increase coronary flow but diminishes cardiac work in patients with coronary heart disease,[7] thereby diminishing the ratio of left ventricular work over left ventricular oxygen consumption. This effect, rather than coronary vasodilatation, has been postulated as the mechanism responsible for the relief of pain in patients with coronary heart disease. The findings presented in this paper make it likely that smoking also exerts its acute effect in coronary artery disease not through an action of nicotine on coronary flow but through its effect on the work of the heart.

References

1. BARGERON, L. M., JR., D. EHMKE, F. GONLUBOL, A. CASTELLANOS, A. SIEGEL & R. J. BING. 1957. Effect of cigarette smoking on coronary blood flow and myocardial metabolism. Circulation. **15:** 251.
2. RUSSEK, H. I., B. L. ZOHMAN & V. J. DORSET. 1955. Effects of tobacco and whiskey on the cardiovascular system. J. Am. Med. Assoc. **157:** 563.
3. DAVIS, F. W., JR., W. R. SCARBOROUGH, R. E. MASON, M. L. SINGEWALD & B. M. BAKER, JR. 1953. The effects of exercise and smoking on the electrocardiograms and ballistocardiograms of normal subjects and patients with coronary artery disease. Am. Heart J. **46:** 529.

4. Levine, S. A. 1958. Clinical Heart Disease. Saunders. Philadelphia, Pa.
5. Gorlin, R. & J. V. Warren. 1958. Hemodynamic Methods—Heart and Lungs. Methods in Medical Research. Yearbook Publ. Chicago, Ill.
6. Michal, G. & R. J. Bing. 1959. Myocardial efficiency. Ann. N. Y. Acad. Sci. **72**(12): 555.
7. Gorlin, R., N. Brachfeld, C. MacLeod & P. Bopp. 1959. Effect of nitroglycerin on the coronary circulation in patients with coronary artery disease or increased left ventricular work. Circulation. **19:** 705.

TOBACCO SMOKING, THE ELECTROCARDIOGRAM, AND ANGINA PECTORIS

Bertil von Ahn
Lasarettet, Eksjö, Sweden

Ever since Favarger in 1887[20] claimed that excessive tobacco smoking produced coronary constriction which, repeated over many years, gradually resulted in organic heart disease, tobacco has been considered an important cause of coronary disease. This view has been generally maintained, especially by German authors up to 1940, including Assmann, Deneke, Plenge, Schönberg, Strauss, Weiker, Lickint, and others.[1] Thus, according to general opinion twenty years ago, tobacco smoking often produced coronary spasm and was a common cause of angina pectoris. Hence it followed that tobacco smoking gradually came to be considered to be a direct precipitating cause of heart infarction. Incorrect interpretation of the electrocardiogram contributed to this viewpoint. Many authorities used to regard a flattening of the T waves alone as evidence of myocardial ischemia, and they therefore considered that the theory of tobacco smoking as a precipitating cause of coronary spasm could be confirmed by the electrocardiogram. If it was indeed true that tobacco smoking could precipitate heart infarction, then smoking was a very severe risk to patients with coronary disease.

Critical voices have been raised, however, suggesting that the supposedly deleterious effect of tobacco smoking has been greatly exaggerated, and that it does not provoke coronary thrombosis (Johnson *et al.*[1]).

Such was briefly the concensus in about 1944. A number of investigations concerning the effect of tobacco smoking on the electrocardiogram in man had already been published at that time.[1] The usual findings during smoking tests were increased heart rate, a slight flattening of the T waves (1 to 2 mm.), and a corresponding slight depression of the S-T segments. Considering the commonly prevailing opinion of the deleterious effect of tobacco smoking on heart activity and coronary flow, these electrocardiographic findings were surprisingly meager: if it were true that smoking produced coronary spasm and angina pectoris, the smoking tests should have provided more concrete evidence of it.

In order to find new facts on the effect of tobacco on the heart, I initiated in 1944 a series of investigations carried out over the years 1944 to 1954. This series comprised a study of the effect of tobacco smoking and nicotine on the electrocardiogram in normal habitual smokers and nonsmokers (published in 1946, 1947) and in patients with severe coronary disease (1949); nicotine-tests on rabbits (1952); a study of two cases of severe acute nicotine poisoning in man (1952, 1953); and, finally, an investigation into the immediate effect of tobacco smoking and nicotine on the electrocardiogram in man during induced hypoxia.[1]

Some details about the pharmacological basis of these experiments will be given first.

Pharmacological Background

According to the pharmacological textbooks, nicotine produces in animals an initial decrease in heart rate through stimulation of the vagus or through paralysis of the sympathetic nerve. Then, after large doses, tachycardia is induced through sympathetic stimulation or through paralysis of the parasympathetic ganglia. Nicotine stimulates both the sympathetic and parasympathetic ganglia, and it influences directly the muscles of the heart and vessels. The effect of nicotine thus varies greatly.

In animals nicotine produces coronary constriction, but is also capable of dilating the coronary vessels (see Laubry *et al.*[1]). Bülbring *et al.* (1949)[21] have shown that in anaesthetized dogs nicotine produces an initial increase in the coronary flow, followed by a prolonged reduction.

Nicotine also stimulates certain endocrine glands, including the adrenals and posterior lobe of the pituitary. Because of this, smoking results in increased liberation of adrenaline, noradrenaline, and antidiuretic hormone (Burn, 1951).[22] This last-named hormone is believed to be identical with pitressin. Burn and Rand have shown that nicotine can release small quantities of noradrenaline and adrenaline from stores within the heart.[2]

The pharmacological effect of nicotine is thus complicated, the net effect being difficult to estimate.

In the past the textbooks of pharmacology have emphasized, often one-sidedly, the constrictor effect of nicotine on the coronary vessels. Most clinicians seem to have shared this view, regarding it as evidence in favor of the hypothesis that tobacco smoking precipitates coronary spasm and angina pectoris. It is a fact, however, that nicotine in small doses is capable of dilating the coronary vessels. This is seldom mentioned in the pharmacological and clinical literature, but is emphasized in Goodman and Gilman's recent textbook.[3]

In ordinary smoking the constant initial finding is tachycardia (stimulation of sympathetic ganglia), in contrast to the bradycardia (parasympathetic stimulation) found in animal experiments. Only in connection with prolonged chain-smoking is marked stimulation of the vagus reflected in the electrocardiogram with bradycardia, high, broad T waves, and an upward displacement (elevation) of the S-T segments.

The doses of nicotine, measured per kg. body weight, generally employed in animal experiments have considerably exceeded the amount of nicotine absorbed during ordinary smoking. On smoking one cigarette, 0.007 to 0.0143 mg. of nicotine is absorbed per kg. body weight in an adult man weighing 70 kg.

If it is true that dilation of the coronary vessels is brought about via the sympathetic fibers and constriction via the parasympathetic fibers, it is reasonable to assume that small doses of nicotine will result in increased coronary circulation due to stimulation of the sympathetic ganglia. To this, however, must be added the direct effect of nicotine on the muscles of the vessels and myocardium, and the increased liberation of antidiuretic hormone and catecholamines.

Electrocardiographic Findings

During the years 1946–1954 a number of papers on the effect of tobacco smoking on the electrocardiogram have been published. These are reviewed in my monograph.[1] The findings may be summarized as follows:

(1) Ordinary tobacco smoking results in an increase in heart rate by 15 to 24 beats/min.

(2) At the same time there is a slight depression of the S-T segments, and a flattening of the T waves by 1 to 3 mm. in leads 1, 2, and 3.

(3) The increase in heart rate and the flattening of the T waves are more pronounced in healthy young subjects than in older persons and elderly, habitual smokers.

(4) The effects of smoking and intravenous injection of nicotine (1 to 2 mg.) on the ECG are identical.

(5) The ECG findings during smoking are essentially similar in healthy persons and in patients with coronary disease.

(6) Many patients with severe heart disease are able to chain-smoke to near intoxication without showing immediate cardiac deterioration.

(7) Angina pectoris never develops in healthy smokers while actually smoking, even after prolonged chain-smoking.

A few patients with coronary disease react with true angina pectoris and simultaneous electrocardiographic changes, both signs of temporary myocardial ischemia.

In these cases tobacco seems to exert a very untoward, not to say dangerous, effect on cardiac function. The coronary reserve remains reduced for several hours, cardiac efficiency is very markedly lowered, and the disposition to anginal attacks increases.

(8) Tobacco smoking or injection of nicotine in man during induced hypoxia produces essentially the same electrocardiographic changes as smoking under normal conditions.

No electrocardiographic evidence of myocardial ischemia can be found in man even during induced hypoxia.

(9) Extrasystoles are seldom recorded on the ECG during smoking.

(10) In no case has heart infarction been reported in connection with smoking tests.

(11) Two cases of transient auricular fibrillation during severe acute nicotine poisoning in man have been described.

(12) In rabbits large doses of nicotine must be given (at least 3.25 mg./kg. body weight) in order to provoke arrhythmia.

Since the publication of these experiments two more reports have appeared (Kreuziger *et al.*[4] and Russek *et al.*[5]). These do not alter the above conclusions concerning the electrocardiographic findings. A third case of paroxysmal auricular fibrillation on acute nicotine poisoning in man was described in Poland by Pedich in 1956.[6]

How may the electrocardiographic changes observed during smoking be explained?

Physiological stimuli such as moderate exercise or emotion produce in healthy subjects an increase in heart rate, linear flattening of the T waves, and lowering of the S-T segments. This is exactly what may be found on the electrocardiogram during smoking. The more the heart rate increases during smoking, the more pronounced is the flattening of the T waves and the S-T segments. The electrocardiographic changes are not so marked as to be considered pathological.

The electrocardiographic changes in smoking can be reproduced by a physiological stimulus (exercise on the bicycle ergometer) or by injection of atropine. They can be totally or partly eliminated by injecting dihydroergotamine.

These facts provide strong evidence in support of the hypothesis that electrocardiographic changes associated with smoking are due to increased sympathetic tone provoked by the stimulus of nicotine on the sympathetic ganglia or by parasympathetic inhibition. The electrocardiographic changes following smoking have been misinterpreted in the past, and their importance has been greatly exaggerated. In assessing the electrocardiogram it is essential to bear in mind any effect of increased heart rate alone and the effect of associated physiological phenomena upon the record. Statistical examination of the findings reveals the effect of nicotine tachycardia on the electrocardiogram to be insignificant[1] (FIGURES 1, 2, 3).

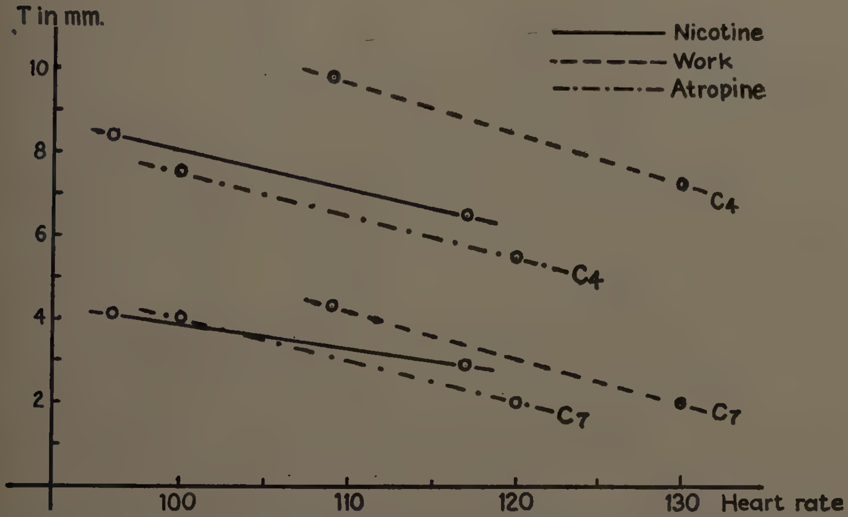


FIGURE 1. Comparison of the T waves after atropine (0.75 mg.), nicotine (1 mg.), and exercise during hypoxia. The lines drawn between the amplitudes of the T waves at 6 and 17 min. of hypoxia have the same inclination in leads CR_4 and CR_7 in all tests. After von Ahn.[1]

In a few cases, after smoking or injection of nicotine in man, I have noted electrocardiographic changes more pronounced than expected, considering the simultaneously increased heart rate. These changes were strikingly similar to those seen after small doses of adrenaline. It is reasonable to assume that in these very cases a particularly great secretion of adrenaline had occurred (FIGURE 4).

Since smoking may also cause increased liberation of antidiuretic hormone (pitressin) from the posterior lobe of the pituitary, there is another possible explanation. Pitressin exerts a constrictor effect on the coronary vessels. Greatly increased secretion of pitressin may also have occurred in isolated cases. This hormone may possibly predispose to myocardial ischemia, especially in hypoxia. Bellet *et al.*,[23] in experiments on animals, have shown that an ischemic heart is particularly sensitive to nicotine.

It is striking, however, that the nicotine effect on the electrocardiogram in man during hypoxia is so slight.

Bülbring *et al.* have compared the effect of nicotine and pitressin on the coronary circulation in animals. From their experiments they conclude that sufficient antidiuretic hormone is liberated on ordinary smoking to reduce the coronary flow in man. In some particularly susceptible subjects the amount

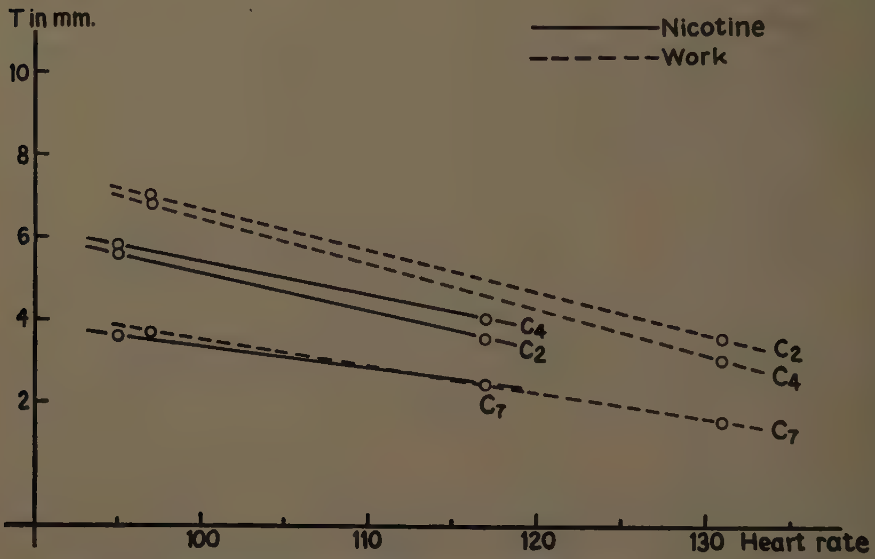


FIGURE 2. The straight lines demonstrate the flattening of the T waves in leads CR_2, CR_4, and CR_7 after exercise and intravenous injection of 3 mg. of nicotine. The nicotine line has exactly the same inclination as the "physiological" line after exercise in the various leads.

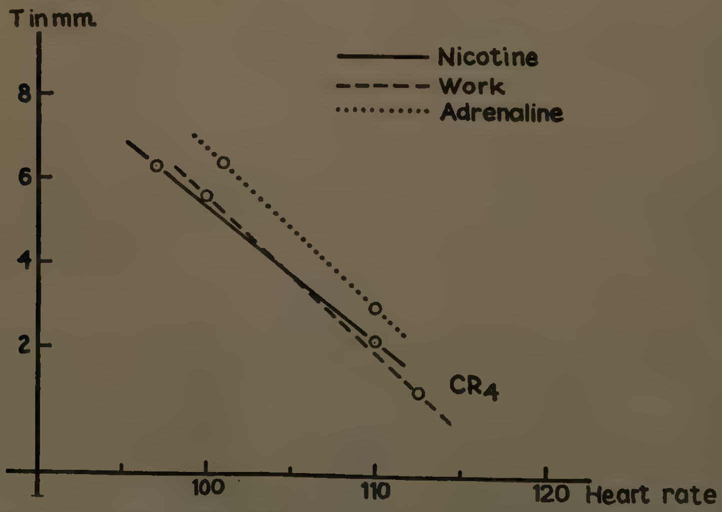


FIGURE 3. The straight lines demonstrate the flattening of the T waves in lead CR_4 after nicotine (0.33 mg.), adrenaline (7.5 γ), and exercise. All three lines have the same inclination, indicating that the electrocardiogram was equally influenced in each of the three tests.

of hormone liberated is calculated to be considerable. On the other hand, Walker[24] observed no antidiuretic effect in 6 of 13 students after the smoking of one cigarette. Five of these six were smokers. Not until another cigarette had been smoked was inhibition of diuresis noted. The simultaneously increased secretion of adrenaline and, particularly, of noradrenaline may possibly have reduced the effect of pitressin on the coronary flow. Adrenaline increases the oxygen consumption of the heart and may predispose to myocardial ischemia, whereas noradrenaline dilates the coronary vessels. However, the amount of pressor amines liberated from the suprarenals on smoking is considered by certain investigators to be so small as to lack any practical importance.[7,8] If this is largely true the effect of pitressin on the coronary flow should predominate.

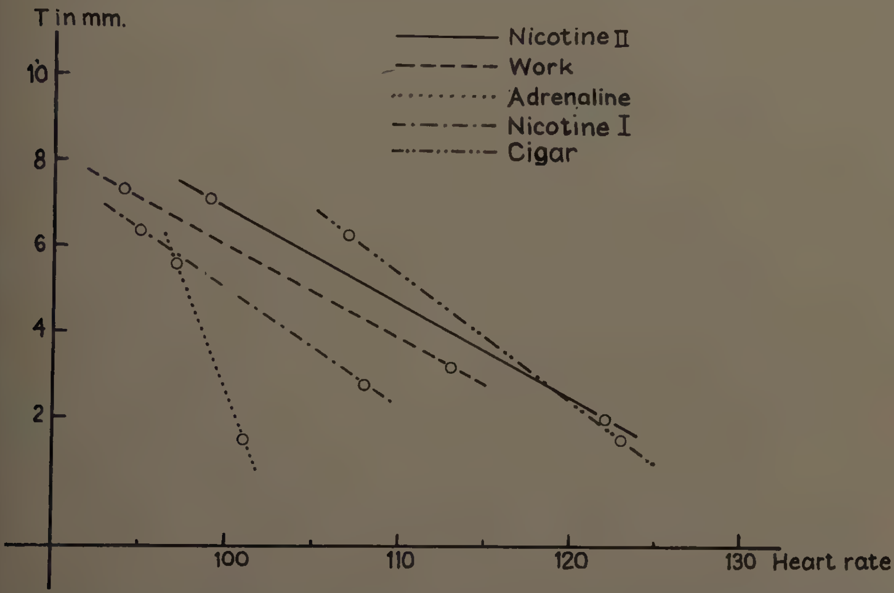


FIGURE 4. Comparison between the flattening of the T waves in lead CR_4 after cigar smoking, nicotine (1 mg. and 3 mg.—nicotine I resp. II), adrenaline (30 γ), and exercise. The lines have practically the same inclination, except for that of adrenaline, which inclines steeply. Thus adrenaline (in the dose employed) exerts a considerably more potent effect on the T waves than do nicotine and exercise.

Bargeron *et al.*[9] published in 1957 an interesting investigation on the effect of cigarette smoking on the coronary circulation in man. Catheterization of the coronary sinus was performed in 14 subjects without known coronary disease. The coronary flow was estimated by "the nitrous oxide desaturation" method. The result showed a significant increase in coronary flow on smoking.

Tobacco Smoking and Chest Pains

It is common knowledge that prolonged and excessive tobacco smoking can provoke chest pains. Huchard gave in his textbook of 1889 (first edition) a particularly graphic account of this syndrome in heavy smokers, and introduced the term "tobacco angina."[1] The etiology of this well-defined syndrome has been much discussed and is still unknown. The pains do not as a rule develop in immediate association with smoking. They are sometimes very severe and of long duration, and often resemble a stubborn neuralgia. The state may possibly be a toxic neuralgia of the Plexus cardiacus, as Richter has proposed.[10] Tobacco angina occurs in clinically healthy smokers as well as in smokers with coronary disease and may, in these latter, be difficult to distinguish from, and may simulate, a heart infarction. It may be added that nitroglycerin has no effect on these chest pains.

I have investigated the influence of smoking on some cases of tobacco angina. None of the subjects developed chest pains, and no ECG changes could be observed during or immediately after the smoking tests.

In some cases, however, smoking immediately precipitates angina pectoris with pathological ECG changes. This never occurs in smokers with clinically healthy hearts, but only in smokers with coronary disease. In these cases there is undoubtedly hypersensitivity to tobacco, as stipulated by Joseph Harkavy,[25] who has studied allergic states in tobacco smoking. This hypersensitivity may take different forms. Smoking may result in immediate tachycardia, or in true coronary spasm with normal or slightly increased heart rate, or both. These two phenomena can easily aggravate a latent myocardial ischemia and precipitate angina pectoris. I have described three cases of true angina pectoris after smoking probably caused by the conditions assumed in this discussion.

It is very rare to find patients with angina pectoris that develops in immediate relation to tobacco smoking. I have been looking for such cases in Sweden over a period of ten years, but have found only these three and, up to 1954, only about a dozen had been described in the literature.[1]

On the basis of the findings of Burn *et al.* on the effect of nicotine on the pituitary, smoking might be supposed to produce a deleterious effect on the coronary flow, especially in patients with coronary disease and small coronary reserve. This does not seem to me to be the case, however. Many patients with severe coronary disease can chain-smoke to the limit of intoxication without showing signs of angina pectoris or other immediate disturbance of cardiac function. Robert Levy *et al.* also subscribe to this view.[11] The effect of pitressin can therefore hardly be very marked in habitual smokers, in whom nicotine is rapidly metabolized. The rapid breakdown of nicotine and its fate in the organism have been studied in detail by Larson *et al.* and Werle *et al.*[12] According to Wolff *et al.*[28] no less than 80 to 95 per cent of the nicotine absorbed during smoking is metabolized during the actual period of smoking.

The differential diagnosis of chest pain is not easy. Such pain includes cardiac pain, rheumatic pain, biliary and peptic ulcer pain, and pain of nerve root origin, to mention only a few. The diagnosis of angina pectoris and even of heart infarction has often been made in cases of radicular syndrome.[13] In the past, before it was realized that there were so many widely differing causes, chest pain in smokers was undoubtedly often ascribed to coronary disease provoked by tobacco (see in particular the German literature, including Strauss, Lickint, and others). Faulty diagnosis has been responsible for the belief that tobacco smoking is a common cause of coronary spasm and true angina pectoris. If tobacco angina occurs in a patient with true coronary disease and angina pectoris, the situation is more complicated and is readily misinterpreted. It may then be very difficult to decide which pains are due to tobacco and which to the coronary disease.

In summary it may be said that electrocardiographic investigations carried out in man have not shown that ordinary smoking has any immediate deleterious effect on the heart, or that it predisposes to angina pectoris, except in rare cases.

Thus the electrocardiographic findings do not support Favarger's old hypothesis of tobacco smoking as a cause of coronary spasm and heart disease.

This does not imply, however, that tobacco smoking in the long run is an innocent habit. Prolonged and excessive consumption of tobacco, especially in the form of cigarette smoking, is generally considered to have a deleterious effect on the cardiovascular system.

Statistical investigations carried out in recent years confirm that heavy smoking may be at least a contributory factor in the genesis of coronary disease.[14,15,16,17] There are, however, conflicting opinions concerning this viewpoint. That a statistical correlation between the smoking habit and coronary disease has been noted does not prove that there is a direct biological relationship between them. It is possible that the relationship is only indirect, as Bronte-Stewart suggests, both the habit and the disease being dependent on "a third and common factor."[18] A similar view has been advanced by Friedman and Rosenman, who observed no "pathogenic relevance" between cigarette smoking and coronary disease.[19]

References

1. von Ahn, B. 1954. The acute effect of tobacco smoking and nicotine on the electrocardiogram especially during induced hypoxia. Acta Med. Scand. Suppl. **292:** 1–112.
2. Burn, J. H. & M. J. Rand. 1958. Action of nicotine on the heart. Brit. Med. J. **I:** 137.
3. Goodman, L. S. & A. Gilman. 1958. The Pharmacological Basis of Therapeutics. Macmillan. New York, N. Y.
4. Kreuziger, H., R. Heinecker & F. Kemper. 1955. Ueber den Einfluss des Zigarettenrauchens auf den Kreislauf. Z. Kreislauff. **44:** 879.
5. Russek, H. I., B. L. Zohman & V. J. Dorset. 1955. Effects of tobacco and whiskey on the cardiovascular system. J. Am. Med. Assoc. **157:** 563.
6. Pedich, W. 1956. Przypadek migotania przedsionkow w nastepstwie ostrego zatrucia nikotyna. (Case of auricular fibrillation as a result of severe nicotine poisoning.) Odbitka Z "Polskiego Tygodnika Lekarskiego" R. XI Nr. 16.
7. Straub, W. & A. Amann. 1940. Ueber die Wirkung des Nikotins und die Diätetik des Tabakgenusses. Klin. Wochschr. **19:** 169.
8. Rehder, K. & G. M. Roth. 1959. Effect of smoking on the fasting blood sugar and pressor amines. Circulation. **20:** 224.
9. Bargeron, L. M., Jr., D. Ehmke, F. Gonlubol, A. Castellanos, A. Siegel & R. J. Bing. 1957. Effects of cigarette smoking on coronary blood flow and myocardial metabolism. Circulation. **15:** 251.
10. Richter, F. 1939. Quoted by F. Lickint, Tabak und Organismus. : 489. Hippokrates-Verlag Marquardt. Stuttgart, Germany.
11. Levy, R. L., J. H. L. Mathers, A. A. Mueller & J. L. Nickerson. 1947. Effects of smoking cigarets on the heart. J. Am. Med. Assoc. **135:** 417.
12. Werle, E., H. Schievelbein & D. Spieth. 1956. Zur Pharmakologie des Nikotins und zur Entgiftung des Nikotins durch den Organismus. Arzneimittelforschung. Mitt. Klosterneuburg. **5:** 265; **6:** 322.

13. Davis, D. 1957. Radicular Syndromes with Emphasis on Chest Pain Simulating Coronary Disease. Year Book Publ. Chicago, Ill.
14. British Medical Journal. 1955. *I*: 91. Smoking and coronary thrombosis.
15. British Medical Journal. 1956. *II*: 90. Smoking and the cardiovascular system.
16. Roth, G. M. & R. M. Shick. 1958. Effect of smoking on the cardiovascular system of man. Circulation. **17:** 443.
17. Buechley, R. W., R. M. Drake & L. Breslow. 1958. Relationship of amount of cigarette smoking to coronary heart disease mortality rates in men. Circulation. **18:** 1085.
18. Bronte-Stewart, B. 1956. Smoking and the cardiovascular system. Brit. Med. J. **II:** 659.
19. Friedman, M. & R. H. Rosenman. 1959. Association of specific overt behavior pattern with blood and cardiovascular findings. J. Am. Med. Assoc. **169:** 1286.
20. Favarger, H. 1887. Die chronische Tabakvergiftung und ihren Einfluss auf das Herz und den Magen. Wien. klin. Wschr. Nr. 11–14.
21. Bülbring, E., J. H. Burn & J. M. Walker. 1949. Reduction in coronary flow by pituitary (posterior lobe) extract in relation to the action of nicotine and to smoking. Quart. J. Med. **18:** 73.
22. Burn, J. H. 1951. Antidiuretic effect of nicotine and its implications. Brit. Med. J. **II:** 199.
23. Bellet, S., A. Kershbaum, R. H. Meade & L. Schwartz. 1941. The effect of tobacco smoke and nicotine on the normal heart and in the presence of myocardial damage produced by coronary ligation. Am. J. Med. Sci. **201:** 40.
24. Walker, J. M. 1949. The effect of smoking on water diuresis in man. Quart. J. Med. **18:** 51.
25. Harkavy, J. 1938. Hypersensitiveness to tobacco and biopsy studies of skin reactions in vascular disease. J. Allergy. **9:** 475.
26. Harkavy, J. 1946. Vascular allergy. Clinics. **5:** 504.
27. Larson, P. S., J. K. Finnegan & H. B. Haag. 1949. Studies on the fate of nicotine in the body. J. Pharmacol. Exptl. Therap. **95:** 506.
28. Wolff, W. A., M. A. Hawkins & W. E. Giles. 1948. The spectrophotometric estimation of nicotine in blood. J. Biol. Chem. **175:** 825.
29. *Idem*. 1949. Nicotine in blood in relation to smoking. J. Pharmacol. Exptl. Therap. **95:** 145.

Part V. Panel Discussion

SIGNIFICANCE OF ELECTROCARDIOGRAPHIC AND BALLISTOCARDIOGRAPHIC CHANGES INDUCED BY SMOKING

Isaac Starr, *Moderator*
University of Pennsylvania School of Medicine, Philadelphia, Pa.

ISAAC STARR: I find myself in a very familiar position. My career has been in the area lying between clinical medicine and physiological and pharmacological sciences, so I have spent my working life suspended between these disciplines. I am sure that my clinical friends think of me as a physiologist or a pharmacologist—I don't know which—but I am equally sure that physiologists and pharmacologists think of me as a clinician, and they would have reason to do so because, for about 25 years, I took charge of a medical ward for at least four months every year; thus, I have had a great deal more clinical experience than most physiologists.

On the other hand, my friends among clinicians are accustomed to say that I do not think the way they do. They tell me that my thinking is like that of the physiologists. I have no doubt that this is true, and I do not think of it as a criticism.

My background being what it is, I plan in this discussion to consider what is said elsewhere in this monograph in relation to certain clinical conceptions and to restate some of the pertinent observations that have been made in recent years.

May I start by pointing out what has been the reaction of the ordinary doctor to smoking. There has been, for example, a strong opinion that smoking is in some undefined way adverse to physical fitness, and that people in training for running races or rowing races or any great exertion or skill performed better if they did not smoke. In so far as I know this has remained an opinion with very little objective evidence to support it, but it is certainly a widely held opinion. I might say, however, that some of the best baseball players are reported to smoke heavily.

Another thing that has long been in the doctor's mind is that tobacco has something to do with extrasystoles. Thus if a 50-year-old gentleman complains of feeling a couple of thumps in his chest for the first time (and practically everybody over 50 has extrasystoles), his doctor is likely to inquire whether he has not been smoking a little too much and to advise him to cut it down. This advice is given because, in my opinion, the doctor does not know what else to say. In so far as I know, there is no objective evidence for the belief that smoking is the cause of extrasystoles or that cessation of smoking is effective in diminishing their frequency.

For a long time the profession has had the knowledge that in some people smoking caused attacks of angina pectoris. When I was in medical school, this tobacco angina was considered to be a great rarity, and if one found a clear case of that kind (and there were indeed clear cases) it deserved reporting in the medical literature. The idea that tobacco has lesser but more frequent effects on the heart is a newer development.

It can also be said with great confidence (and I am sure Burn is pleased with this) that adrenalin will cause anginal attacks in those subject to this disease. There is no doubt about this. Indeed, a good and wise clinician once suggested that an injection of adrenalin provided a good test for the detection of angina pectoris and, for a while, many doctors gave adrenalin to their patients as a diagnostic test; if an anginal attack followed, the diagnosis was proved. I can assure you that such attacks often did follow adrenalin and, in my part of the world, some of these were so severe and alarming that the test had to be abandoned.

I must admit that I do not know whether noradrenalin will cause angina or not, but I can remind you that when, after patients have had a severe attack of cardiac infarction and the blood pressure has fallen to alarming levels, noradrenalin has been used very frequently. Indeed the general clinical opinion is that noradrenalin is probably the best of the drugs now known to combat that situation. This situation is a very serious one, and many of these patients have died. This drug does not seem to cause pain in these cases, and certainly it appears to benefit many patients.

I ought also to speak of the new data from England indicating a very strong correlation between the smoking habits of doctors and their deaths from coronary heart disease. This observation is, to my mind, one of the factors in the situation that cannot be ignored.

Finally, I ought to point out that the classic clinical tests, the pulse and heart sounds, provide no evidence of the association of angina pectoris with the heart. It was in my medical lifetime and that of the older people here that the famous clinician Clifford Albut doubted whether the pain in angina pectoris came from the heart. He thought it came from stretching the aorta. You must also remember that the clinical recognition of cardiac infarction, and the association of that pain with the heart, also came during my medical lifetime. In my early career I was taught that this pain was an "acute indigestion" that sometimes resulted in death in some obscure fashion. Accordingly it is evident that the simple clinical methods such as listening to the heart and feeling the pulse fail to detect a great deal of heart disease that can be detected by modern methods.

Frank W. Davis, Jr. (*The Johns Hopkins University School of Medicine, Baltimore, Md.*): Interest in smoking and the ballistocardiograph has grown largely from efforts by my associates and myself to devise a means of introducing stress to the cardiovascular system that might be measured and monitored in patients who have actual coronary heart disease or potential coronary heart disease; that is, some means of further delineating the apparently normal population from the population with coronary disease—but a method that still makes ballistocardiographic recording possible.

We have undertaken a wide variety of stressful procedures using the ballistocardiogram as a monitor. The stresses we used have included anoxemia and exercise, smoking, nicotine, and ergonovine. To date our best stress test, used

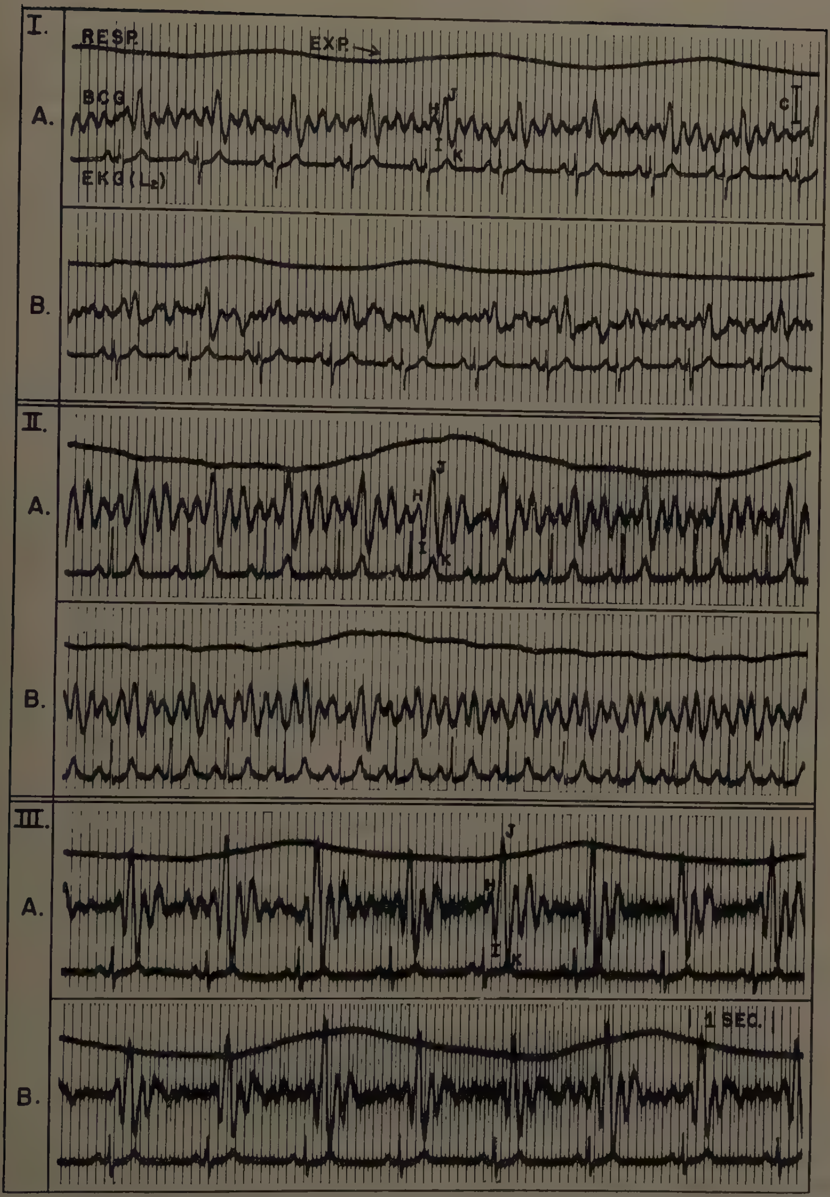


Figure 1. The effects of cigarette smoking on the ballistocardiogram. (IA) Control record of a 51-year-old man with angina pectoris. (B) BCG immediately after smoking. The amplitude decreases, the H wave shows relative augmentation, and I and J are shortened and rounded. This represents a positive test. (IIA) Control BCG from a 50-year-old man with angina and hypertension. (B) BCG immediately after smoking. Many "early-M" complexes appear. This is also a positive test. (IIIA) Control BCG from a healthy 55-year-old physician. (B) Record immediately after smoking, showing no change from the control BCG. This is a negative test. Reproduced by permission of C. V. Mosby Co. from the *American Heart Journal*, **46**: 529.

in an empirical manner, has been the ballistocardiogram observed before and after smoking a cigarette.

In simple terms, we have found that the most useful approach is simply to obtain a control ballistocardiogram, electrocardiogram, and a blood pressure measurement, ask the subject to smoke a cigarette and observe the changes as they occur immediately after smoking and five minutes after smoking. The reaction is classified as negative if there is no change in the ballistocardiographic pattern, as borderline if there is deterioration of expiratory complexes, and as positive if there is gross deterioration of the ballistocardiographic pattern.

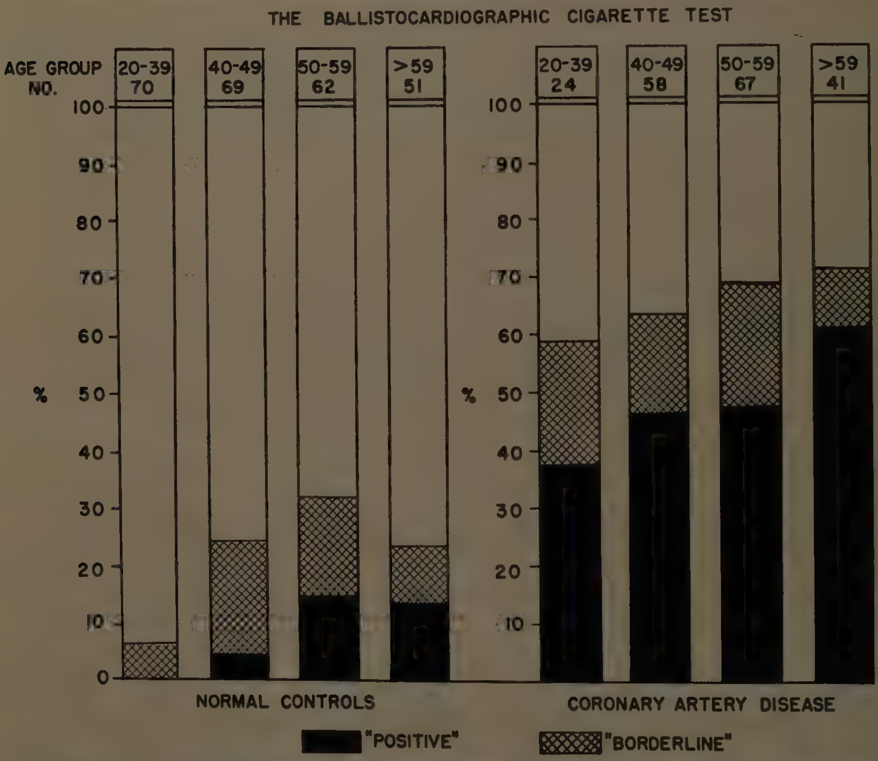


FIGURE 2. The incidence of borderline and positive ballistocardiographic cigarette tests in normal subjects and patients with coronary heart disease. The columns represent decades (the first column in each group represents two decades), with the number of subjects presented at the top of each column. (The differences in incidence of positive tests between normal and diseased groups is statistically significant in each decade.) Reproduced by permission of C. V. Mosby Co. from the *American Heart Journal*, **51**: 165.

FIGURE 1 shows representative responses from three subjects. For each a control BCG and an immediate postsmoking BCG is shown. The first two are from males in the sixth decade with angina, and the gross change from the control to the distinctly altered pattern after smoking may readily be seen. In contrast, a control and postsmoking BCG in a normal male physician of the same age group shows no change. These are examples of what we consider positive and negative responses.

Many readers of these pages are perhaps familiar with our results in studying large numbers of patients and controls using this test. We have made well over 1000 such tests thus far, but I now propose to show older data obtained from the first 450 subjects thus studied and to review briefly some of the results obtained.

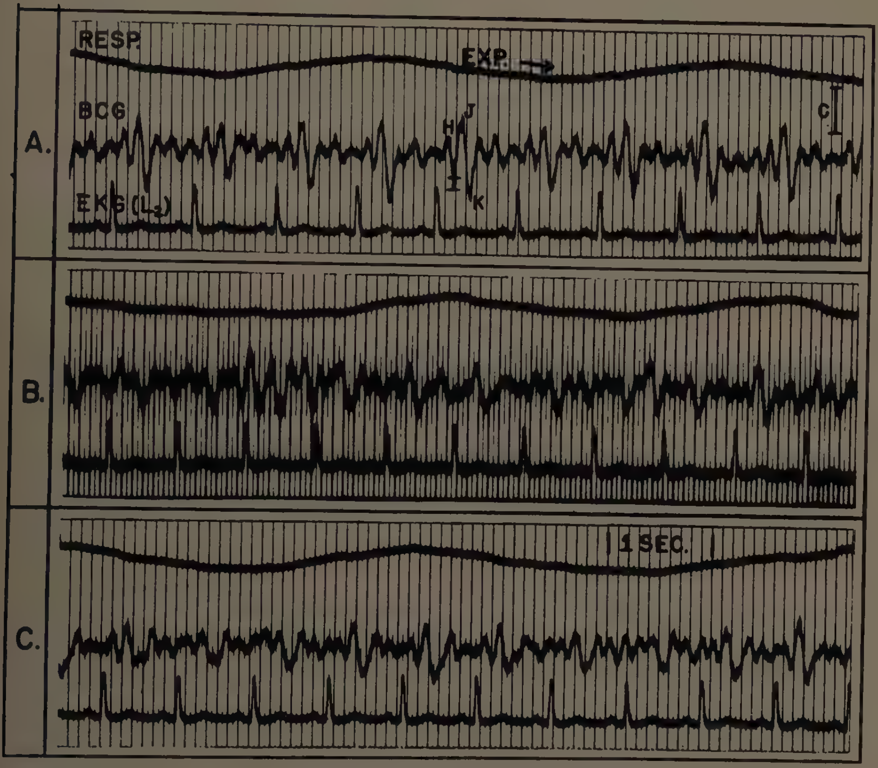


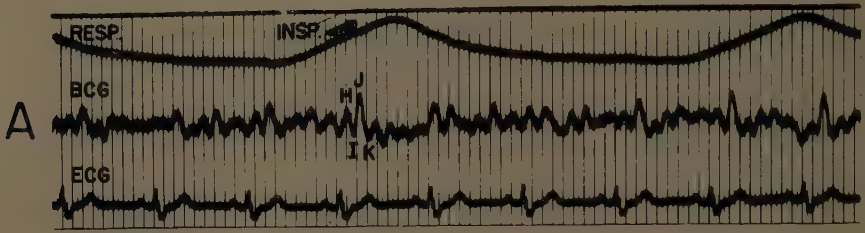
FIGURE 3. The effects of oral nicotine on the ballistocardiogram in a 53-year-old man with angina pectoris. (A) Control BCG. (B) BCG immediately after smoking. (C) BCG after the sublingual administration of 0.5 mg. nicotine base in 0.1 mg. doses over a 5-min. period. Note the general similarity to the postsmoking record. Reproduced by permission of C. V. Mosby Co. from the *American Heart Journal*, **46:** 529.

FIGURE 2 shows the percentage of positive cigarette tests by age decade in the normal controls and in patients with coronary heart disease. Without belaboring the minor features of this figure, several points are obvious. The first is that in the normal controls there are no positive tests in subjects under the age of 40. We have yet to see a positive test in a normal subject under 40. There is an increasing incidence of positive tests with increasing age in the normal control group, an over-all incidence of about 7 per cent positive tests in normal controls. Most of these subjects are over 50 and are predominantly males.

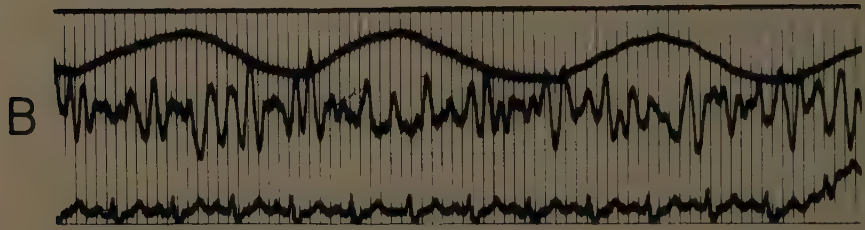
In contrast, in the patients who have clinical coronary heart disease, a positive response was obtained in approximately one half the subjects. There is a similar age relationship but it is not quite as striking as in the normal controls.

The diagnostic value of this test still exceeds that of any other instrumental method used in our laboratory.

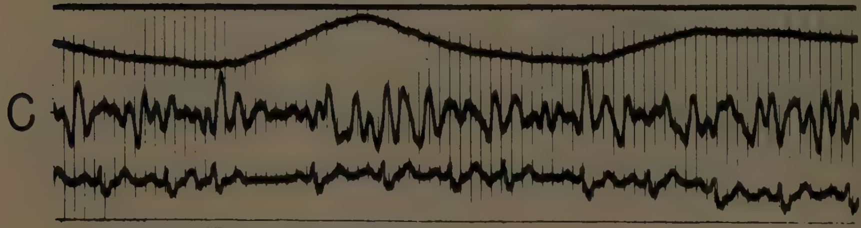
To evaluate further the means by which these changes are brought about we have administered nicotine by several routes to the patients with coronary heart disease. We first administered sublingual nicotine in small aliquots of 0.1 mg. of nicotine base per minute over a 5-min. period in an attempt to approximate the absorption obtained in smoking.



CONTROL



IMMEDIATELY p̄ 1.0mg. NICOTINE I.V.



5 MINUTES p̄ 1.0mg. NICOTINE I.V.

FIGURE 4. The effects of 0.1 mg. of nicotine base administered intravenously in a 64-year-old man with angina pectoris. Control tracing is abnormal, but immediately and 5 min. after nicotine there is gross deterioration of the complexes. There is an element of respiratory artifact.

FIGURE 3 shows ballistocardiograms from a male subject with angina. The effect of smoking is demonstrated by the change from a slightly abnormal to a grossly abnormal pattern. The same subject, when given nicotine sublingually, showed similar although perhaps less striking changes.

In all the subjects in whom sublingual nicotine has been administered we noted changes roughly comparable to those obtained with smoking.

In some subjects the changes were more striking with sublingual nicotine, while in others they were more striking with cigarettes.

In order to evaluate further the nicotine effects, we have recently undertaken a study of intravenous nicotine monitored with the ballistocardiograph. Intravenous nicotine seems perhaps more variable, at least to date, than sublingual nicotine, for reasons that are not clear to me, but in a dosage that varies from 0.5 to 1 mg. of nicotine base we were usually able to achieve BCG changes comparable to those obtained with smoking.

FIGURE 4 presents the results of a representative intravenous nicotine study in a 65-year-old male with angina. This patient's control pattern is abnormal. His immediate postnicotine record (1 mg. nicotine base given intravenously over a 1-min. period) shows striking alteration from that seen in the control period. Some of these alterations are respiratory artifacts, but there is gross alteration in the pattern obtained in mid-inspiration. The gross changes from the previous BCG persist 5 min. after the intravenous nicotine.

May I mention briefly several other features that I think are important in terms of the effect of cigarettes on the ballistocardiographic changes observed in our laboratory. First, we used a wide variety of filtered cigarettes, filtered cigarette holders, denicotinized cigarettes, and low nicotine cigarettes; irrespective of the method used to try to avoid these changes, all cigarettes and filters have failed to protect the sensitive subject from these reactions.

This, I think, is not surprising in view of the fact that only very small amounts of nicotine seem to be necessary in the sensitive coronary heart disease subject to bring about BCG alterations. We could prevent these changes in most instances by the presmoking administration of nitroglycerin, or we could abolish them after they had occurred.

One other thing that seems important to us is that it makes little difference whether the patient inhales or does not inhale. It makes little difference whether he is a smoker or a nonsmoker.

One more facet that I shall touch upon briefly is the question of whether this test has any prognostic addition to diagnostic significance. At the moment we have not completed the study of the past 10 years of cigarette tests; but I may say that as a prognostic method this test seems largely limited in usefulness to the young. As a matter of fact, the test as a whole seems most useful in the young.

Of the patients during the past ten years who have had coronary heart disease from the first, and who subsequently died, all who died under the age of forty had a positive cigarette test. Among the over-all group of coronary patients who died or who survived, 66.6 per cent of those who died had positive tests at some time during the period of study. This is not very impressive, because 50 per cent of the entire group had positive tests.

BÖRJE EJRUP (*Karolinska Sjukhuset, Stockholm, Sweden*): Many early investigators were of the opinion that the changes that appeared in the electrocardiogram in connection with smoking or after injection of nicotine were the result of a coronary spasm, and that this explained the patient's symptoms. Others pointed out the importance of the autonomic nervous system in this phenomenon. The results of von Ahn's (1954) investigations refuted the theories of coronary spasm as the cause and confirmed the role of the autonomic nervous system. His thesis emphasized the importance of Nordenfelt's (1941) work with ergotamine and amyl nitrite to demonstrate the functional changes in the electrocardiogram, also of Sjostrand's (1950) demonstration of the relation between heart rate, S-T level, and changes in the T wave of the electrocardiogram. These observations are fundamental in the interpretation of ECG changes in smokers as well.

Von Ahn (1954) also carried out investigations under accentuated test conditions, including hypoxia, during which patients attempted to smoke and tried exercising. By means of atropine, Adrenalin, and ergotamine experiments, von Ahn demonstrated that the ECG changes were pulse-rate dependent. The higher pulse rate after smoking he regarded as a sign of stimulation of the sympathetic nervous system and considered it scarcely probable that any coronary spasm was present. Only in isolated cases was von Ahn able to induce ECG changes due to smoking that indicated deterioration of coronary circulation, that is, in three patients with coronary sclerosis.

TABLE 1

REASONS GIVEN BY 1012 PATIENTS (616 MEN, 396 WOMEN) FOR WISHING TO STOP SMOKING

Reason	No. of patients
Poor physical condition	562
Nervousness	524
Chronic bronchitis	461
Stomatitis	418
Cardiac or vascular symptoms	240
Digestive symptoms	240
No. of patients who claimed they were unable to refrain from smoking for even one day	735

In 1946 von Ahn also carried out experiments with chain smoking of up to 22 consecutive cigarettes and, in several other cases, until the patient reached a state of intoxication, but he was unable to demonstrate any ECG changes other than those due to the more rapid heart rhythm.

My own investigations are of an entirely different nature. They include studies of electrocardiograms from patients with chronic nicotine intoxication from smoking. Electrocardiograms have been recorded at rest and after different amounts of exercise up to the individual maximal load. As a rule the examination has subsequently been repeated after three weeks' abstinence.

Subjects

In connection with the work now being carried on in Stockholm on breaking the smoking habit, data have been collected on symptoms associated with smoking from a comparatively large number of persons (about 3000). A more thorough analysis of the smoking habits, the effectiveness of the treatment, the reasons for the patient's smoking, and why he or she wants to stop smoking, has been carried out on a group of 1012 persons (Ejrup, 1956, 1959).

TABLE 1 gives some data from that study.

As is evident from the table, 562 of 1012 patients, that is, more than half, wanted to stop smoking because of poor physical condition; 240, or about one fourth, wished to stop because of cardiovascular symptoms. It is also interesting to note that chronic bronchitis was present in one half of the cases. On the whole all the aforementioned symptoms disappeared when the patients stopped smoking. The general character of the group is indicated by the fact that it included 735 persons who felt they could not be without tobacco for a single day. On the average they smoked a package of 20 cigarettes per day.

Against the background of the symptoms reported, one should expect to find electrocardiographic changes or other signs of cardiac damage from smoking. My experience with this group, however, showed that such was not the case.

Different Investigations of the Subjects

The first group electrocardiographically investigated consisted of 80 smokers, who all complained of real or suspected symptoms from the cardiovascular system.

Only five patients presented pathological ECGs. In 19 cases extrasystoles were found. All of the 80 cases stopped smoking after being treated at our antitobacco weaning clinic. In none of the five cases with signs of organic changes of coronary insufficiency was there any reason to suspect the smoking, and the changes persisted unchanged during abstinence.

The remainder of the group had completely normal ECGs, and no later routine test or control ECG was taken. The patients' symptoms disappeared after the treatment, and obtaining control ECGs at that time presented considerable practical difficulty.

This part of the investigation showed that no significant ECG changes were found clinically, notwithstanding the patients' noticeable subjective symptoms. Only a few exhibited extrasystoles, which wholly conforms with the experience of other investigators (von Ahn, 1946).

Another electrocardiographically examined group consisted of 28 patients selected at random from the clinic.

Before and after the treatment they underwent tests of the blood, X-ray examination of the heart and lungs, and ECG at rest and after 8 min. in a standing position. Special heart function tests were carried out on an ergometer bicycle, according to Wahlund's (1948) method, and three ECGs were made, one every two minutes, using routine methods worked out in the Pathophysiological Department of the Karolinska Sjukhuset.

The entire series of tests was then repeated at a reexamination three weeks later, a fortnight after stopping smoking.

In a third group of 18 patients, some of the lung functions were examined before and after induced abstinence.

Method of Investigation

(1) After 20 min. of initial rest, the patient was placed in a reclining position and examined with standard leads (leads 1, 2, and 3), unipolar extremity leads (aVR, aVL, and aVF), and chest leads (CH_1 to CH_7, with the exception of CH_3).

(2) After eight minutes in an upright position, the patient was examined with the same leads.

(3) While sitting on the ergometer bicycle, the patient was then examined

electrocardiographically during the second, fourth, and sixth minutes of exercise equivalent to 200 kpm./min.* for women and 300 kpm./min. for men. Leads 1, 2, 3, and CH_4 were used.

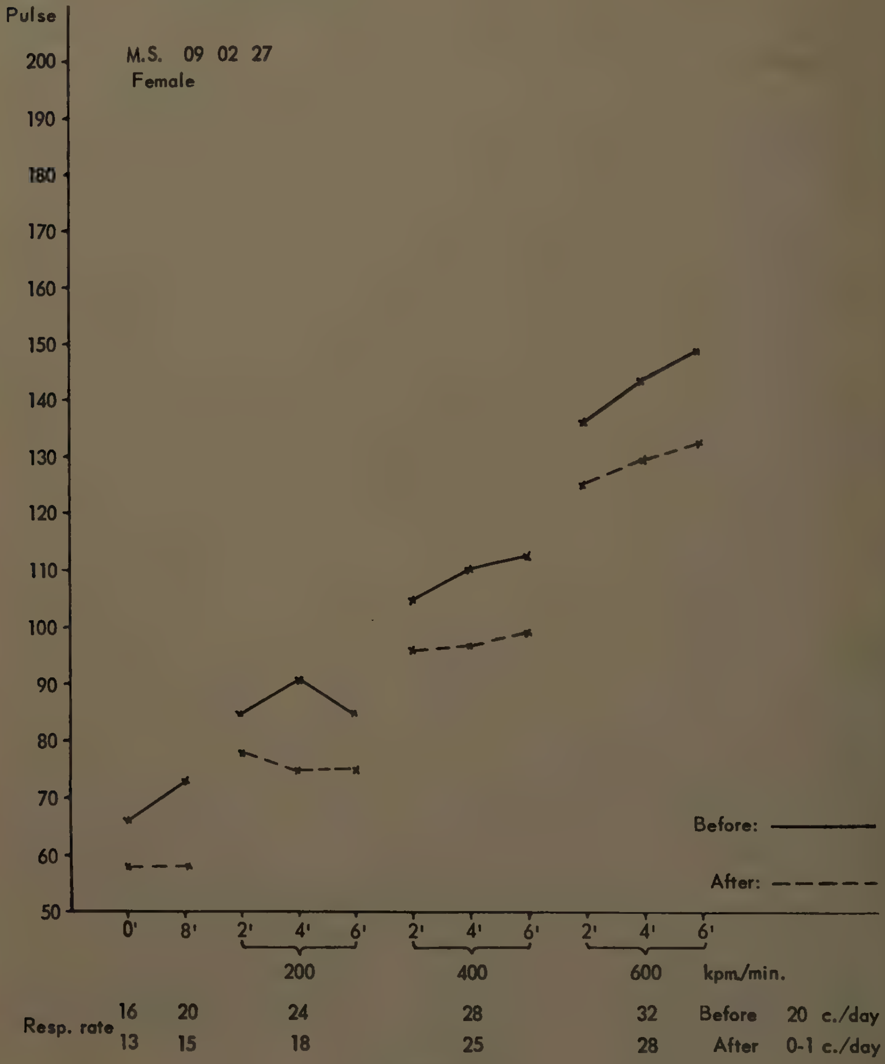


FIGURE 1.

(4) Immediately after this examination, the loads were increased to 400 and 600 kpm./min., respectively, and new electrocardiograms were recorded during the second, fourth, and sixth minutes of the more strenuous exercise.

(5) Three new electrocardiograms were made, at the same intervals, but with the loads increased again to 600 and 900 kpm./min., respectively.

(6) If the patient was able to continue further, the loads were then increased

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* Kilogram-meters/min.

to 800 kpm./min. for women and 1200 kpm./min. for men, and the tests were repeated.

The respiratory rate was recorded during each test. The pulse rate was read on the electrocardiogram.

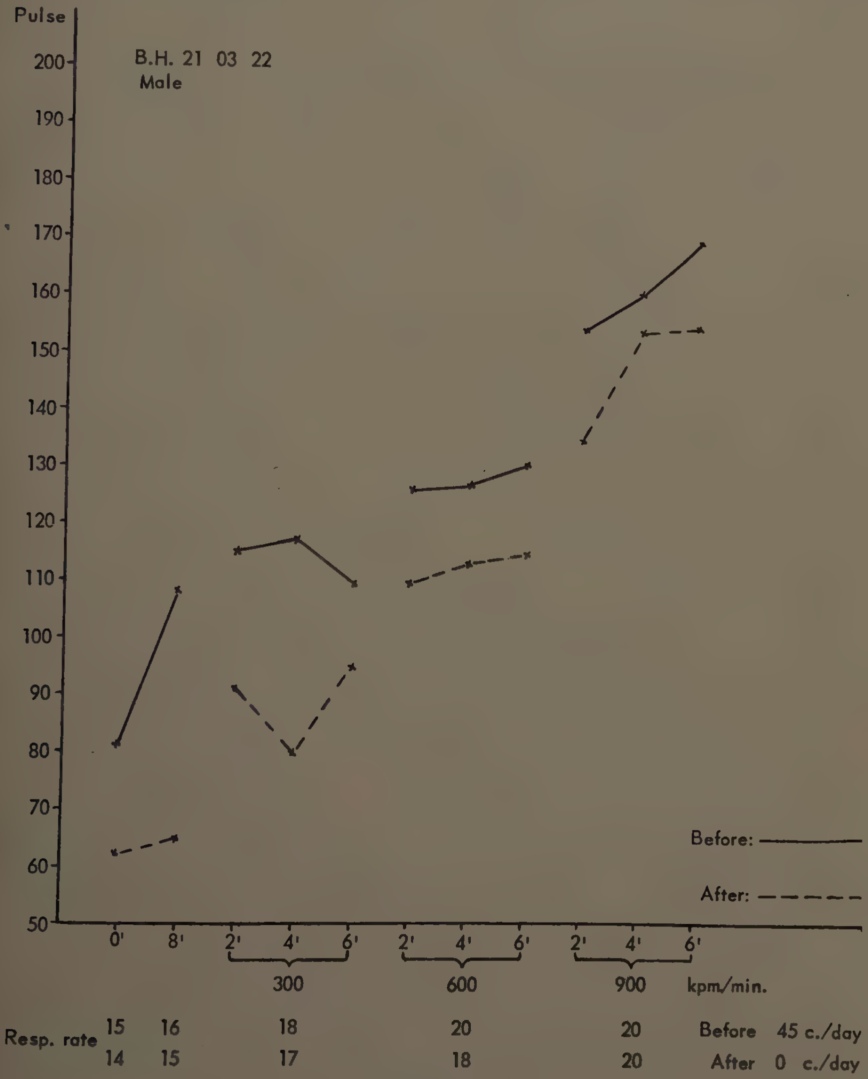

FIGURE 2.

Results and Discussion

FIGURE 1 shows the results of such a series of tests on one patient. The vertical scale presents the pulse rate, as measured on the electrocardiogram, and the horizontal scale indicates the type and magnitude of the work performed. The point at the extreme left of the figure shows the pulse rate at rest, and the second point from the left indicates the pulse rate after eight minutes in an upright position. The pulse rate after 2, 4, and 6 min. is shown for each work load. The solid line represents the result obtained before the cure and the broken line, the result obtained one week after finishing the cure, or during abstinence.

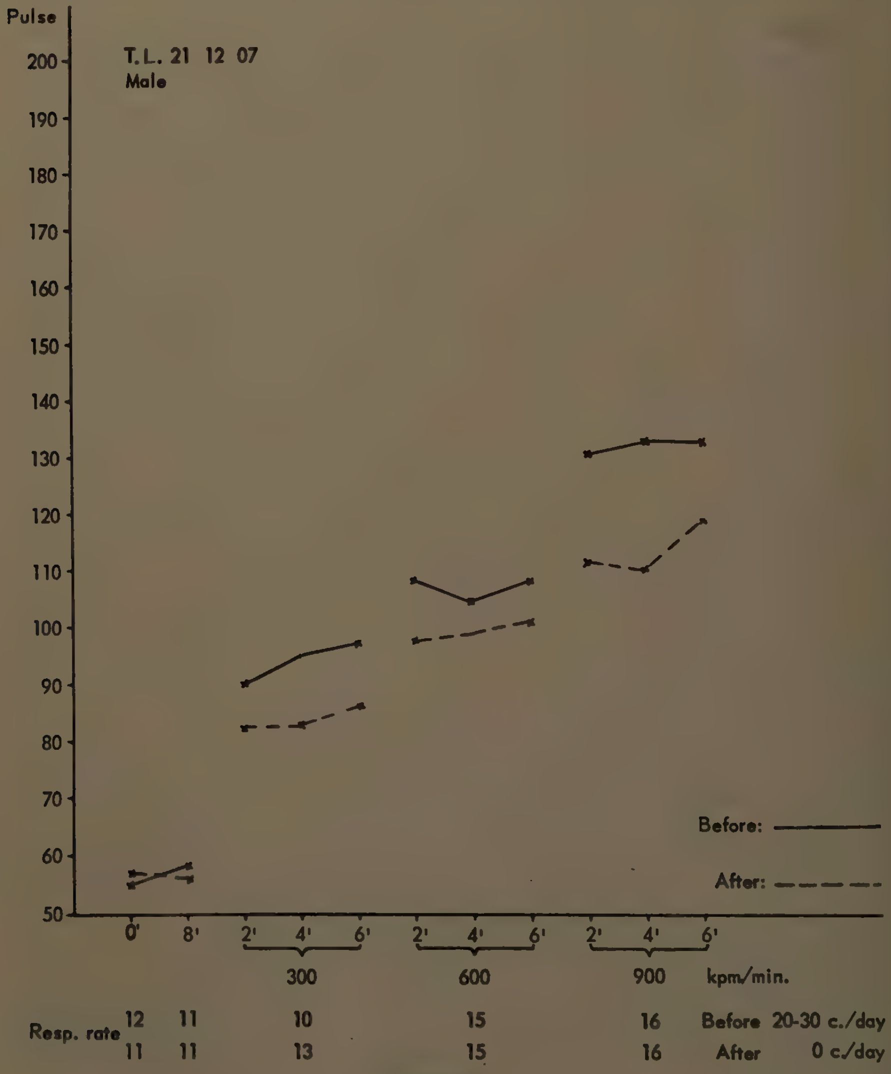

FIGURE 3.

At the bottom of the figure the respiratory rate before and after the cure is presented.

As a rule the patients smoked from 20 to 40 cigarettes per day before the cure and no cigarettes one week after the cure. In four or five cases, however, the patients smoked two or three cigarettes per day after the cure.

As may be seen from the data in FIGURES 1 and 2, in patients at rest, then for eight minutes in upright position, and later under the different work loads, there is a difference in the pulse rate, with a higher rate before, and lower after, the cure.

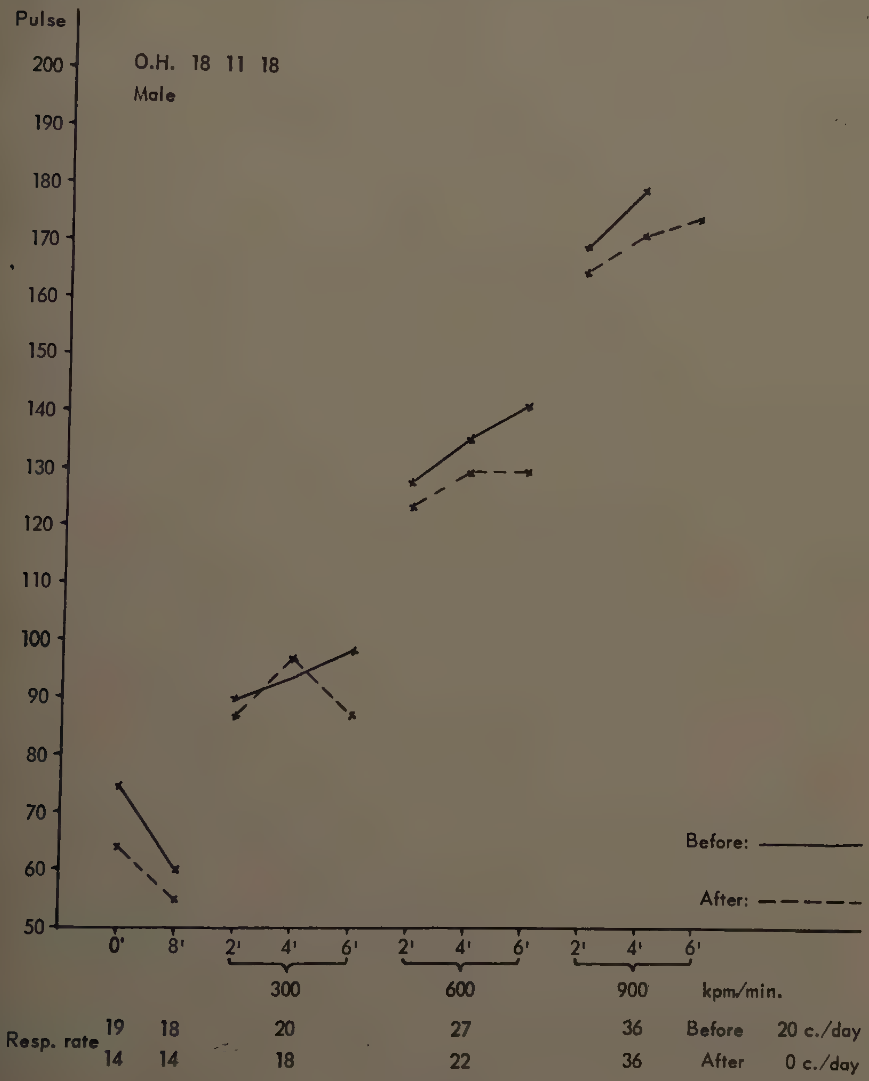

FIGURE 4.

In FIGURE 3, we find the same relationship. The difference is especially great in the upright position. Without cigarettes the pulse was 65; during the time when the patient was smoking the pulse rate was 108.

FIGURE 4 presents results obtained from an athlete in good training. His curve exhibits a lower pulse rate in general with increased magnitude of the work load; after stopping smoking, however, a still lower pulse rate was obtained.

On the other hand, the next case, that of a 35-year old woman who smoked 20 cigarettes per day before the cure and cut down to from two to five per day after the cure, we find no significant difference between the two function tests (FIGURE 5).

As may be seen from TABLE 2, of the 28 individuals chosen at random for the study, 13 improved, eight were unchanged, and seven declined, considered in terms of pulse rate. In terms of respiratory rate, however, 18 improved, three were unchanged, and six worsened.

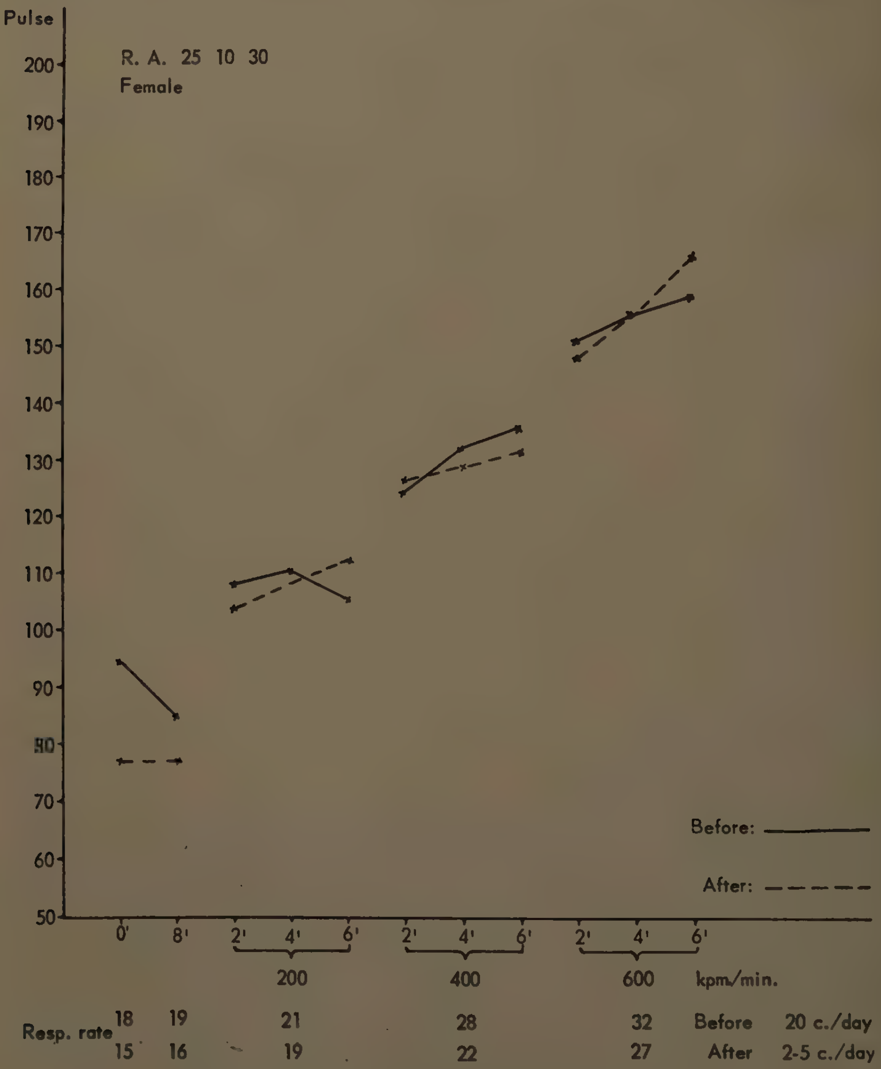

FIGURE 5.

One expects that patients exhibiting tachycardia during a period of smoking should be worse off functionally and should more easily and quickly reach the point of insufficiency. The difference in the heart rate before and after the cure, however, is not particularly great, and in some cases no difference was found at all. If there were an increased risk of coronary spasm, it should be apparent on the electrocardiograms recorded during the more strenuous exercise. Such is not the case except in a few instances; nevertheless it is important that these instances should not be completely ignored.

TABLE 2

PULSE AND RESPIRATION BEFORE AND AFTER ABSTINENCE

Case no.	Initials	Sex	Age	Pulse rate result*	Respiratory rate result*
1	A.H.	F	60	I	I
2	A.A.	F	54	NI	W
3	E.R.	M	53	W	I
4	G.K.	M	52	W	I
5	T.B.	M	52	W	I
6	M.S.	F	51	I	I
7	E.S.	M	51	W	I
8	R.W.	M	51	NI	I
9	S.W.	M	48	I	W
10	B.T.	F	46	I	W
11	E.A.	M	46	NI	I
12	T.K.	M	46	W	W
13	G.S.	M	44	NI	I
14	C.L.	F	42	I	I
15	O.H.	M	42	I	I
16	G.N.	M	41	I	I
17	M.L.	M	41	W	—
18	N.B.	M	40	NI	NI
19	B.H.	M	39	I	I
20	T.L.	M	39	I	NI
21	C.C.	M	36	W	I
22	L.H.	F	36	I	I
23	R.A.	F	35	NI	I
24	E.T.	M	35	NI	NI
25	S.S.	M	34	I	W
26	B.B.	M	33	NI	W
27	R.H.	M	28	I	I
28	J.G.	M	27	I	I
			Totals: (for 21 Males and 7 Females)		
			I:	13	18
			NI:	8	3
			W:	7	6

* Comparisons were made of the subjects' pulses and respiratory rates before and after giving up smoking. I is an improvement; NI indicates no significant improvement; W means worse.

An investigation with a similar method but without electrocardiograms used on 40 men in compulsory military service, carried out by Hirvonen *et al.* (1960, personal communication) should be mentioned in this connection. Work performed at a heart rate of 170 beats per minute was taken as basal value (according to the suggestion of E. Bengtsson, 1956).

The Finnish investigators found that after the smoking of two cigarettes the function measured in kpm./min. decreased 9.6 per cent ± 1.15 per cent.

The Study of the Electrocardiograms

In the function tests I carried out, as mentioned earlier, electrocardiograms were recorded at all the points indicated in the figures, and the examination of these ECGs also included study of the S-T segments and T waves. The majority of the patients showed S-T depressions during the more strenuous exercise and the T waves decreased in height. In some cases, a certain difference was apparent in the electrocardiograms before and after the cure, in that the S-T segment was somewhat more depressed before, that is, while the patient still smoked. This difference rarely exceeded 1 mm. in the extremity leads and 2 mm. in the chest leads, however. Those changes could be correlated with an increased heart rate, according to Sjöstrand (1950, 1952), and not necessarily regarded as inducing coronary spasm.

TABLE 3
LUNG FUNCTION OF SMOKERS BEFORE AND AFTER ABSTINENCE

Case no.	Initials	Sex	Age	Cigarette consumption/day		FEV in 1 sec. as a % of FVC*		Results†
				Before	After	Before	After	
1	A.H.	F	60	15	0	91.5	91.2	NI
4	G.K.	M	52	13	0	58.6	72.3	I
9	S.W.	M	48	40	0	83.4	83.6	NI
12	T.K.	M	46	30	0	90.7	87.0	W
14	C.L.	F	42	20	0	66.7	80.0	I
15	O.H.	M	42	20	0	71.7	77.1	I
16	G.N.	M	41	10‡	0	66.7	73.7	I
20	T.L.	M	39	25	0	86.2	87.8	I
23	R.A.	F	35	20	4	63.8	63.4	NI
26	B.B.	M	33	20	0	84.1	80.5	W
27	R.H.	M	28	23	0	90.0	93.1	I
28	J.G.	M	27	25	0	82.4	92.4	I
29	H.L.	M	43	30	5	54.4	72.5	I
30	N.K.	M	55	20	0	67.8	74.6	I
31	S.O.	M	63	33	6	69.4	73.8	I
32	A.H.	F	50	20	0	79.6	83.8	I
33	S.L.	M	28	35	0	71.2	87.3	I
34	E.C.	M	56	25	2	83.0	77.1	W

Totals: (for 14 Males and 4 Females)
I: 12
NI: 3
W: 3

* The forced expiratory volume (FEV) of the lungs in 1 sec., expressed as a percentage of the total forced vital capacity (FVC).

† I represents an improvement; NI indicates no significant improvement; W means worse.

‡ Cigars.

In the majority of the cases in this study I have pressed the patients to the point of "coronary insufficiency" with depression of the S-T segments, but have not been able to detect any significant difference in the electrocardiograms before and two weeks after the cure. It should be noted, however, that after the patients have stopped smoking, they were able, as a rule, to drive themselves further and thus were able to perform a somewhat greater amount of exercise with greater subjective ease.

It is desirable that the investigation be extended to include functional tests at later dates; for example, after two months or one year of complete abstinence from smoking. It is possible that a more definite difference will then be apparent in the functional capacity of the patient.

Comparison of the objective findings with the appreciable subjective relief and improvement in condition reported by the patient after he had stopped smoking shows that the functional impairment during smoking must be of a type other than that which can be recorded electrocardiographically.

Some Investigations of the Lung Function

In a third group, consisting of 18 patients selected at random, the lung function was examined in a Bernstein spirometer. The forced vital capacity of the lung (FVC) and the forced expiratory volume in one second (FEV: 1 sec.) were estimated. Twelve of these 18 cases were thoroughly examined, as described for the group of 28 patients mentioned above.

The results are shown in TABLE 3. If the FEV as a percentage of FVC is higher after abstinence than before, I have called it an "improvement (I)." If the difference is less than 1 per cent, I have used the expression "not improved (NI)" and if it is less after abstinence than before, the result is indicated as "worse (W)." Considering the group in these terms, I found 12 cases improved, three not improved, and three worse.

I also investigated the forced vital capacity (FVC) in these cases and observed a definite difference, with a change for the better during the abstinence period. It is possible that ease of breathing is the subjectively important aspect. Diffuse bronchial stenosis with difficulty in expelling the air is interpreted by the patient as an impairment of the physical condition. A more difficult breathing process, involving increased work for the patient, naturally constitutes a handicap, and the essential effect seems not to lie in the cardiovascular system and is not demonstrable electrocardiographically.

References

VON AHN, B. 1954. The electrocardiogram in tobacco smoking during hypoxia. Acta Med. Scand. **148:** 101–121.

VON AHN, B. 1946. En elektrokardiografisk undersökning av tobaks—rökens inverken pa hjårtat hos olika grader av rökare. Svenska Läkartidn. **43** (50) : 3109.

BENGTSSON, E. 1956. The working capacity in normal children, evaluated by submaximal exercise on the bicycle ergometer and compared with adults. Acta Med. Scand. **154:** 91–109.

EJRUP, B. 1956. Försök till avvänjning frän tobak medelst injektionsbehandling. Svenska Läkartidn. **53**(41): 2634.

EJRUP, B. 1959. Rökavvänjningspoliklinikerna. Sv. Läkartidn. **56**(27): 1899.

EJRUP, B. & P. A. WIKANDER. 1959. Fortsatta försök till avvänjning från tobak medelst injektionsbehandling. Svenska Läkartidn. **56**(28): 1975.

EJRUP, B. & P. A. WIKANDER. 1959. Försök med nikotin, lobelin och placebo. Svenska Läkartidn. **56**(29): 2025.

EJRUP, B. 1959. Follow up av ett material svårbehandlade rökare. Svenska Läkartidn. **56**(33): 2254.

NORDENFELT, O. 1941. Über Funktionelle Veränderungen der P- und T-Zacken im Elektrokardiogramm. Acta Med. Scand. Suppl. 116–120.

SJÖSTRAND, T. 1950. The relationship between the heart frequency and the S-T level of the electrocardiogram. Acta Med. Scand. **138**(111): 201–210.

SJÖSTRAND, T. 1950. Experimental variations in the T-wave of the electrocardiogram. Acta Med. Scand. **138**(111): 191–200.

SJÖSTRAND, T. 1952. After potentials in the electrocardiogram. Acta Physiol. Scand. **24:** 247–260.

WAHLUND, H. 1948. Determination of the physical working capacity. Acta Med. Scand. Suppl. 215. Thesis.

John J. Kelly, Jr. (*Cardiovascular Pulmonary Center, Mercy Hospital, San Diego, Calif.*): It is now 10 years since a young physician working as a Research Fellow in Dock's laboratory was discovered to have an abnormal ballistocardiogram after smoking a cigarette, but a normal record at other times. Other examples of this behavior were encountered. Investigation clearly revealed the relationship of this phenomenon to smoking.[1] These observations were presented and little thought was given to this phenomenon until our peace of mind was shaken by Henderson[2] working in Starr's laboratory, and by Davis and his associates.[3] These investigators pointed out the rarity of the conversion of a normal ballistocardiogram to an abnormal one by tobacco in the young and the high frequency of this alteration in subjects with coronary artery disease. This phenomenon clearly results from the nicotine of the cigarette. As Davis indicated earlier, nicotine can produce all of the ballistocardiographic changes seen with smoking a cigarette. The cigarette habit is not necessary for the development of the abnormal pattern, nor is the inhalation of tobacco smoke. We have observed positive ballistocardiographic smoking tests in nonsmokers with coronary artery disease. We have also observed snuff altering the ballistocardiogram in a similar manner in other individuals.

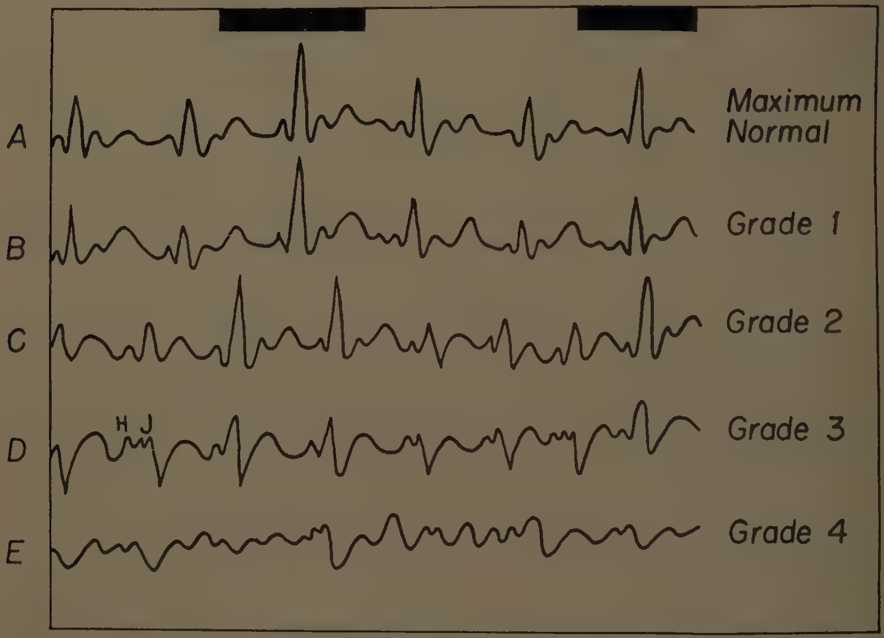

Figure 1. Maximum normal respiratory variation in the ballistocardiogram, *A*. The smaller complexes denote expiration, and inspiration is marked by the black bars. *B* indicates Grade 1 abnormal variation; *C* indicates Grade 2 abnormal variation; *D* is Grade 3; and *E* is Grade 4. *D* also shows fused H-J and notched J waves during expiration. Reproduced by permission from Dock *et al.*, *Ballistocardiography.*[8]

We obtained our traces with a Dock ballistocardiograph. The Brown classification was used to grade these ballistocardiographic records. This system is illustrated in FIGURE 1. We have considered the alteration of a normal record to a grade 3 or 4 as a positive test.

It has been known for some time that both the administration of nicotine and smoking will usually result in an increase in the heart rate and the blood pressure. Peripheral blood flow falls secondary to peripheral vasoconstriction. When investigating the ballistocardiographic smoking test, it would seem wise to determine whether positive tests are seen in subjects who react excessively to nicotine in terms of pulse rate and blood pressure. Experience from our laboratory and others has shown that the response in heart rate and blood pressure is the same in subjects with normal and abnormal smoking tests. This point is illustrared in the following figures. FIGURE 2 is a record obtained on a healthy 17-year-old male. The pulse curve was obtained by means of an indwelling needle in the brachial artery leading to a Statham strain gauge. Under control conditions the subject's heart rate measured 80/min. and blood pressure was 110/64 with a mean pressure of 87. After smoking a standard brand cigarette the pulse rate had increased to 100/min. and the blood pressure to 115/86 with a mean pressure of 98 mm. Hg. The systolic complexes of the ballistocardiogram in both situations are normal. There is a slight increase in the variation of size of these complexes with respiration after smoking. This is a normal response.

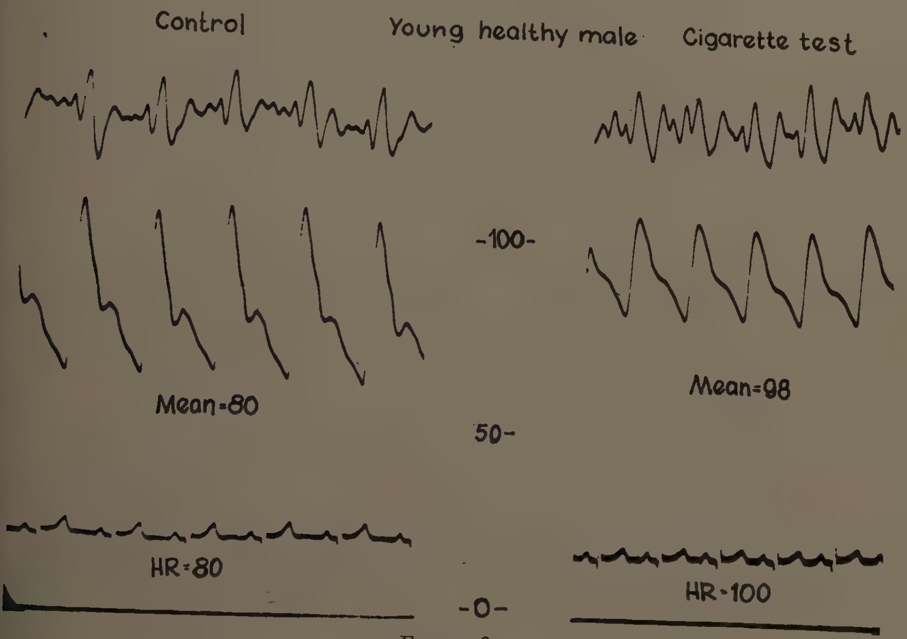

FIGURE 2.

FIGURE 3 illustrates the ballistocardiogram, the brachial artery pressure pulse, and the electrocardiogram obtained under control conditions of a 40-year-old man with classical angina pectoris. The control ballistocardiogram and electrocardiogram are normal.

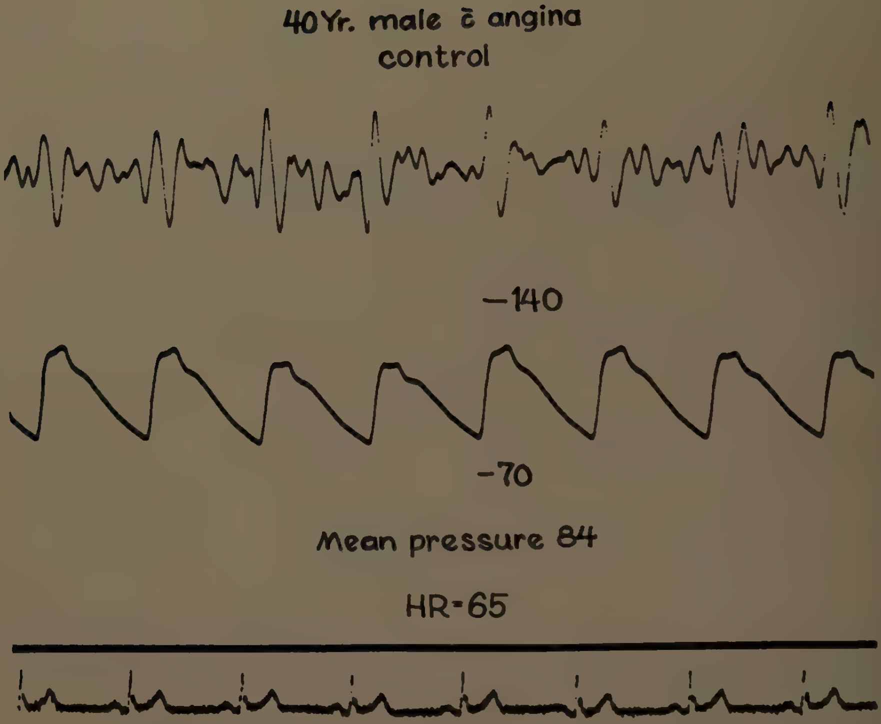

FIGURE 3.

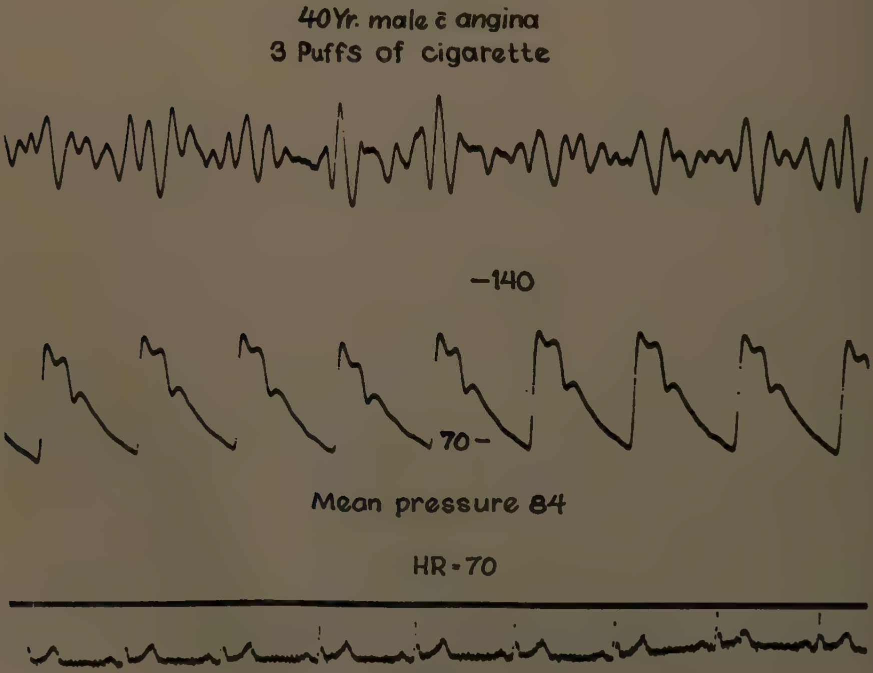

FIGURE 4.

FIGURE 4 is a record on this subject taken 40 sec. after he had begun to smoke, when 3 puffs of a standard brand cigarette had been inhaled. There has occurred no significant change in heart rate or blood pressure, yet ballistocardiographic abnormalities are already present. No change can be seen in the electrocardiogram. This illustrates the rapidity of the action of smoking and the very great sensitivity of the ballistocardiogram to the effects of tobacco. At the completion of the cigarette another record was obtained, which is presented in FIGURE 5. This ballistocardiogram is grossly abnormal; the systolic complexes are distorted and can be rarely identified. A modest increase in blood pressure and pulse rate has occurred, but much less than in the healthy boy.

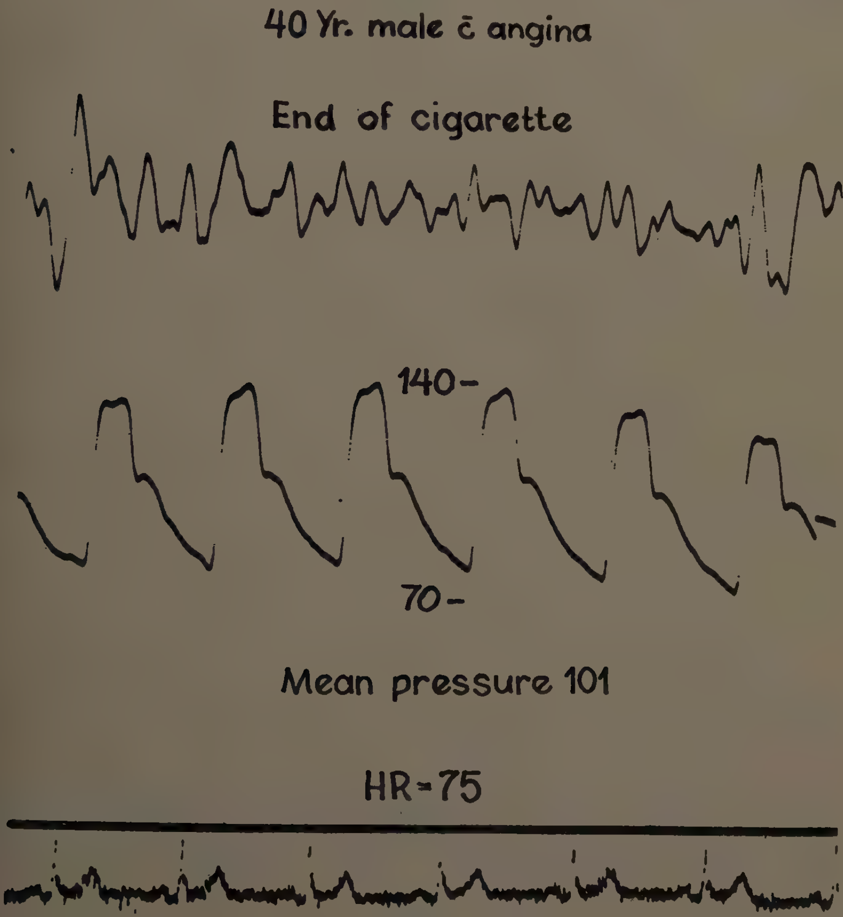

FIGURE 5.

Nitroglycerin will often prevent the deterioration of the ballistocardiogram by smoking. In the instance presented in FIGURE 6, nitroglycerine failed to convert this subject's abnormal record to normal. The reason for the failure in this instance is probably related to the persistence of the high pulse rate and the increased peripheral resistance. When nitroglycerine reduces peripheral resistance by vasodilation, improvement of the ballistocardiogram occurs. This improvement of the ballistocardiographic record with vasodilation is probably due to a decrease in the work of the heart.

Will an increase in peripheral vascular resistance convert a normal ballistocardiogram to an abnormal one in healthy subjects and in subjects with positive smoking tests?

An attempt to answer this question was made with the following experiments.

The blood pressure response to smoking in 10 healthy young subjects and 10 patients with abnormal smoking tests was measured. After blood pressures of both of these groups had returned to control values norepinephrine was administered intravenously until the blood pressures equaled or slightly exceeded those seen with smoking. In no case was there found the familiar ballistocardiographic alteration seen with smoking. It would seem from these observations that an increase in peripheral vasoconstriction alone does not account for the alteration of the ballistocardiogram. Infusion of norepinephrine does not mimic the physiological effects of nicotine in all respects. Both will raise the blood pressure by increasing the peripheral resistance, but nicotine increases the heart rate while norepinephrine usually slows it.

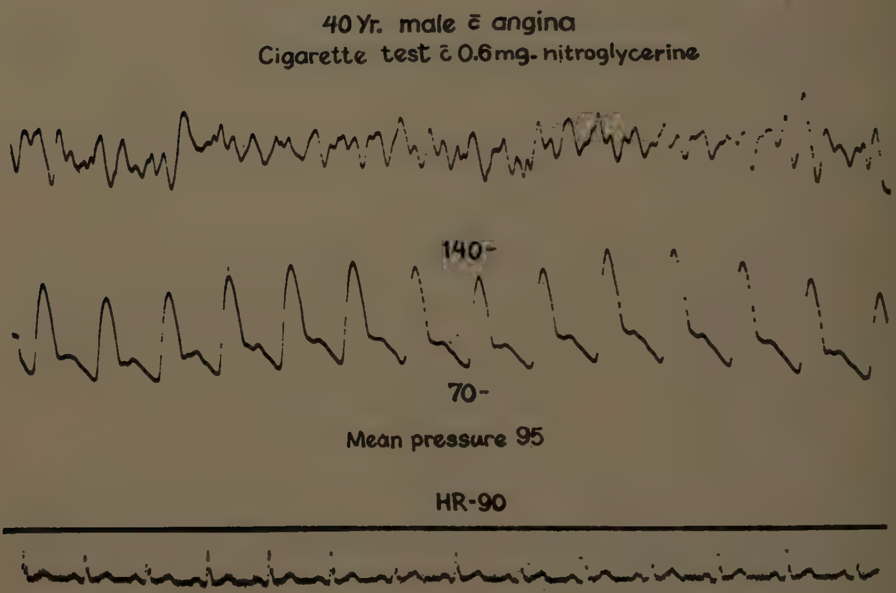

FIGURE 6.

The effect of smoking on the ballistocardiogram has been explained as secondary to the increase in peripheral vascular resistance.[4] These investigators reported that positive smoking tests can be prevented by whiskey. Our experience is meager, but we failed to prevent the development of a positive cigarette test in 4 subjects with coronary artery disease who had been given

2 ounces of whiskey. The whiskey produced excellent vasodilation of the skin vessels in these patients. I believe that Davis and his associates have reported similar experience in this respect.

The evidence presented by others in this monograph provides a convincing argument that nicotine causes a release of catecholamine both in the heart and in the peripheral vessels. The released catecholamine operating at these sites quickens the rate of the heart beat, increases the force of contraction of the myocardium, and causes vasoconstriction in the peripheral vessels. The work of the heart is therefore increased by smoking. Smoking then offers a convenient stress test of the heart. My speculation is that under these conditions anoxic portions of the myocardium contract ineffectively. This is manifested by the ballistocardiogram, which is a cardiac force record and thus extremely sensitive to variations in velocity of ejection of blood from the heart.

I wish to recall the data Davis presents elsewhere in these pages. You might remember a figure in which the frequency of positive smoking tests was related to age in normal subjects. This showed that positive cigarette tests were rare in normal subjects under 40, and increased in frequency with age thereafter. This experience has been confirmed by many others since then. Strober has studied the greatest number of subjects.[5] He examined over 2700 United States Air Force personnel. He too found an increasing incidence of positive smoking tests with age in an apparently healthy population. Strober further noted a relation of positive tests to obesity. Of 120 obese soldiers 45 per cent exhibited a positive smoking test, whereas only 3 per cent of the nonobese group of the same age had reacted with a positive smoking test.

There are several good reasons why the control ballistocardiogram should vary with age. We know that cardiac forces are less well transmitted through the body as the body tissues age. With aging, bowing of the aorta from dilation occurs that results in a change in the vector of cardiac forces; hence less force is recorded in the conventional head-foot plane.

These factors do not, in my opinion, explain the increasing frequency of positive smoking tests with age. A more likely explanation resides in the relationship of coronary artery disease to age. This relationship is well illustrated in FIGURE 7, which was obtained from an exhaustive study of Lober.[6] Lober graded the severity of atherosclerosis in carefully studied coronary arteries of a large number of patients who died from disease other than coronary sclerosis. It is apparent from consideration of these data that atherosclerosis begins in infancy and progresses throughout life in this Minnesota population. The disease does not reach a degree of severity sufficient to cause symptoms in most cases until the 40s or 50s. There is a remarkable similarity between this relation of age and severity of atherosclerosis to other studies relating the incidence of positive cigarette tests to age.

The cigarette ballistocardiographic test has been extremely useful to me as a clinician. I have been greatly impressed by the demonstration of its superiority in the diagnosis of atherosclerosis, compared with the electrocardiographic exercise tolerance test in subjects under 50 years of age.[3] The experience of my associates and myself confirms this careful study by Davis and his group. The ballistocardiogram is more sensitive to myocardial ischemia than is the electrocardiogram. This has been demonstrated by recording simultaneously both the ballistocardiogram and the electrocardiogram in subjects breathing various low-oxygen mixtures. Invariably the ballistocardiogram shows alteration sooner than does the electrocardiogram.[7]

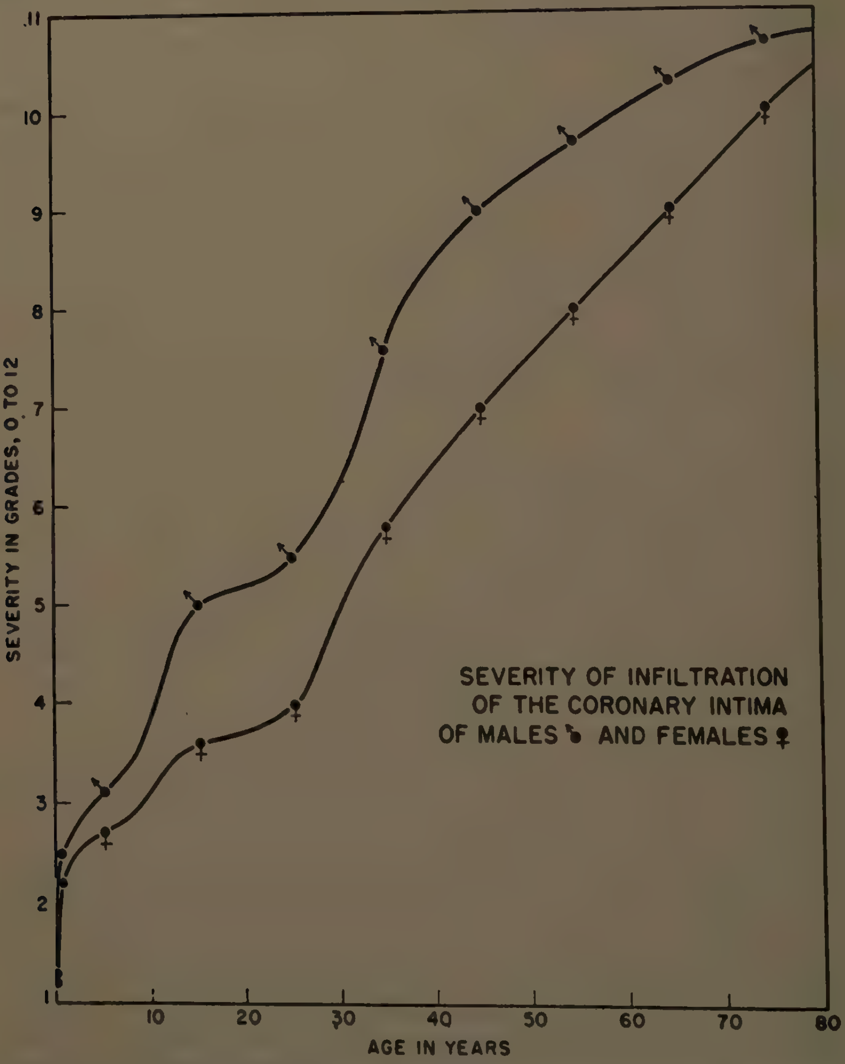

FIGURE 7. Severity of atherosclerosis in males and females as related to age. Reproduced by permission from *A. M. A. Archives of Pathology.*[8]

I have found both the ballistocardiogram and the cigarette test very helpful in following patients recovering from myocardial infarction. It is particularly valuable as a measure of therapeutic response. In patients who have shown good recovery there has been a parallel improvement of the ballistocardiogram, although the electrocardiographic scar might remain. The technique of ballistocardiography is simple and the cost modest. It therefore deserves more attention than it is currently receiving from the physician. Ballistocardiography provides an assessment of cardiovascular behavior that is lacking in the more elaborate cardiovascular function tests.

References

1. CACCESE, A. & A. SCHRAGER. 1951. The effects of cigarette smoking on the ballistocardiogram. Am. Heart J. **42:** 589.
2. HENDERSON, C. B. 1953. Ballistocardiograms after cigarette smoking in health and in coronary heart disease. Brit. Heart J. **15:** 278.
3. DAVIS, F. W., JR., W. R. SCARBOROUGH, R. E. MASON, M. L. SINGEWARD & B. M. BAKER, JR. 1956. The ballistocardiographic cigarette test: further observations. Am. Heart J. **51:** 165.
4. RUSSEK, H. I., B. L. ZOHMAN & V. J. DORSET. 1955. Effects of tobacco and whiskey on the cardiovascular system. J. Am. Med. Assoc. **157:** 563.
5. STROBER, M. 1956. Effect of the dock cigarette-smoking test on the ballistocardiogram: survey of a normal male population. J. Am. Med. Assoc. **161:** 1059.
6. LOBER, P. H. 1953. Pathogenesis of coronary sclerosis. A. M. A. Arch. Pathol. **55:** 357.
7. PENNEYS, R. 1956. Oximeter-controlled induced anoxemia test for coronary artery disease. Trans. Assoc. Am. Physicians. **69:** 214.
8. DOCK, W., H. MANDELBAUM & R. A. MANDELBAUM. 1953. Ballistocardiography. Mosby. St. Louis, Mo.

DAVID SIMON (*Cincinnati General Hospital, Cincinnati, Ohio*): As you may remember, I presented data on patients who chewed tobacco and compared their ballistocardiograms with those who smoke pipes and cigars. We found a very high incidence of ballistocardiographic changes in a similar age group of men with chewing tobacco, but not quite as high with pipes and cigars.

We assume the patients were normal if no changes in pattern resulted. I ask of anyone acquainted with this concept: What is involved in a normal ballistocardiogram improvement?

DAVIS: I think it is fair to say that if a normal ballistocardiogram can improve, some do improve after smoking, both in the normal patients and in some with coronary disease. However, by improvement all we can say is that the amplitude increases. I do not think I have ever seen an abnormal form become normal, but in some normal patterns we may have a higher amplitude.

LOUIS N. KATZ (*Michael Reese Hospital, Chicago, Ill.*): May I ask Davis if he ever had a cigarette made that contains no tobacco, and what effect it has on the ballistocardiogram.

DAVIS: I understand one is being tried in the Midwest at the moment. It is not legal in Maryland; accordingly I have not been able to get this cigarette.

ALDO N. CORBASCIO (*University of California Medical Center, San Francisco, Calif.*): I am interested in Davis' statement about the improvement occasionally seen with nicotine, an improvement that is restricted to one parameter of the ballistocardiogram, the amplitude. This would tie in with what we have seen in animal experiments.

CAROLINE BEDELL THOMAS (*The Johns Hopkins University, Baltimore, Md.*): May I ask Davis what experience he has had in 3-dimensional ballistocardiographic smoking tests. Do all dimensions become deformed at once? Or do some improve whereas others do not?

Davis: I am sorry to hear that question because we really have not studied the 3-D bed after smoking. We have done a number of studies on the 2-D bed with head, foot, and lateral studies in the ultralow-frequency as well as on the high-frequency 2-D bed. I am disappointed to say that for reasons not clear the newer ultralow-frequency methods do not show the changes that we see in the Starr high-frequency bed. Hence I really cannot comment on what the lateral or antero-posterior changes are, since we really do not see very much in the head-foot plane in the ultralow-frequency tracings.

I think it may be that we are working with an artifact due to the body frequency in the Starr high-frequency bed, which is brought out by smoking and not brought out in a pure bed; consequently, this test is best performed on the older bed rather than the new ones.

Morton L. Levin (*Roswell Park Memorial Institute, Buffalo, N. Y.*): In a healthy population is there any difference in the incidence of abnormal BCG responses related to the smoking habits of these people? Or is this merely a predictor of the state of the coronary vessels?

Kelly: There is no difference; that is, previous use of tobacco is not necessary for this phenomenon to appear.

Walter Redisch (*Goldwater Memorial Hospital, New York, N. Y.*): When you say that these people, documented with coronary sclerosis, have improved, I take it to mean that on their tests they show less changes in the ballistocardiogram after smoking. Is that right?

Kelly: They show both clinical improvement in their effort tolerance and in their ballistocardiographic record.

Redisch: Has their myocardium become less sensitive to the release of catecholamines?

Kelly: I think that more probably it represents the growth of new muscle and the development of collateral circulation, rather than any change in the release of the catecholamines.

Davis: May I say a few words in reply to Redisch's question. We have been most impressed with certain therapeutic groups in which striking changes have occurred over a period of years under therapy, in the smoking test as well as in the resting BCG, and I think the possibility of increasing collateral circulation exists. I also think something happens to the viscosity of blood under some of these circumstances. For instance, the changes in resting pattern and also in the postsmoking pattern occur too fast under some circumstances.

I think that when we alter viscosity we may alter the response to smoking. Thus it may not all be changes in the total coronary bed, but it may be something more closely related to acute dynamic differences.

Starr: I shall comment on that because I think we are all being forced to look at coronary heart disease in a much more physiological way than was the case in the past. What has happened, I think, is this. The cardiologists attend the autopsy when the patient dies of heart disease and discover, naturally, that the coronary arteries show structural changes in a very, very high percentage of cases of angina pectoris; and that is what is in the books at the present time.

However, when a 65-year-old man dies in an automobile accident, a cardiologist is not likely to attend that autopsy. If he did he would discover a great deal of coronary sclerosis very similar to that of patients diagnosed as having coronary disease. Thus there is a tremendous amount of overlapping of the anatomical findings. I think we are going to have to make our distinctions on a physiological basis as well as on an anatomical one.

Richard J. Bing (*Wayne State University, Detroit, Mich.*): I shall make two comments. In the first place, we have in this monograph a series of papers on the changes in coronary blood flow, and I think that, perhaps with one exception, no one has found any decrease in coronary blood flow from nicotine or smoking. Here we find marked changes in the ballistocardiogram and it is extremely difficult, as Davis pointed out, to relate these physiologic changes to the ballistocardiogram changes. Changes in viscosity may play a part but we do not know.

With reference to catecholamines in the heart muscle, we have found no diminution in the heart *in vivo* following nicotine.

Starr: One must remember that ballistocardiographic records are records derived from cardiac force, and that very few doctors have begun to think of the heart in the terms of its forces.

There is every reason to think that the cardiac forces are important. For example, what happens when your automobile begins to feel badly? Suppose one cylinder is not firing. The weakened car certainly gets you home all right. It still can go 50 mph on the parkway, but it will not accelerate when the light turns from red to green. In other words, it is the lack of acceleration, and therefore in the weakened forces, that the first evidence of abnormality is likely to be manifested. I believe, of course, that this is going to prove to be true of the heart also.

Question: All three discussants seem to limit themselves to the ballistocardiogram in the discussion of the effects of smoking. I was wondering if anyone on the panel felt there was any marked incidence of standard electrocardiogram changes with smoking, and in what way this might be important.

Ejrup: After smoking there are changes in the standard leads and in the chest leads, but they are related to the rate.

Davis: Occasional patients show striking ECG changes during the smoking test. In our experience between 1 and 2 per cent of subjects have shown changes that we felt were significant. On three occasions very striking ST segment changes occurred in the ECG during a smoking test. This is obviously extremely rare despite the fact that many of these patients have quite severe angina, but there have been several in the first thousand patients we examined.

Ejrup: In cases of severe angina pectoris, it has been found that there are changes in the electrocardiograms that correlate well with a coronary insufficiency. In normal persons we have not seen this, and even in coronary disease it is rare.

Starr: I think that perhaps Bing's question ought to be commented on now, since we have gone a little deeper into the subject. You remember that he raised the question of why the ballistocardiogram so often becomes abnormal after smoking while so many other measurements of cardiac function showed nothing. I think there are two points to be remembered here. In the first place, there is a very marked age difference that is one of the striking features of the field. We do not see these adverse ballistogram changes after smoking in young people. It is only in the older groups that we see them. Perhaps there is an explanation here that will explain the discrepancy between our findings and some of Bing's.

CHARLES J. KENSLER (*Boston University, Boston, Mass.*): I must ask Davis what were the small doses of nicotine that he used. I seem to remember 0.5 mg. to 1 mg. Is that correct?

DAVIS: That is correct. The sublingual tests were all 0.5 mg.; the intravenous doses ranged between 0.5 and 1 mg., the 0.5 mg. being administered over a one-min. period and the 1 mg. over a 5-min. period.

KENSLER: You got the same results when the material was administered sublingually as by intravenous injection?

DAVIS: I cannot say that they are the same but, by and large, I think the changes we get on sublingual or intravenous administration are generally comparable with what we get with smoking, even with this dose of nicotine.

KENSLER: The point I was interested in making here is that intravenous injection of 1 mg. is a large dose compared to smoking, because one is putting one half the yield of a whole cigarette into a person within 5 min. intravenously and building up, I suspect, a considerably higher concentration than occurs in smoking.

In the intravenous situations do you normally control or did you control this test by the injection of saline or some inert material to rule out ancillary effects? Second, can these effects be duplicated by epinephrine? I know Kelly said levoarterenol did not do it.

DAVIS: We have not as yet administered enough intravenous nicotine to have done a control series. We did conduct a control series of the sublingual tests using the same carrier without nicotine. The obvious necessity for doing controls with the intravenous tests still exists. We are just at the moment doing intravenous nicotine studies. We have not tried epinephrine. We have done a few norepinephrine studies and agree fully with Kelly's observations.

STARR: May I say that there is a bit of a hazard in such investigations that any doctor would find it necessary to keep in mind. In the smoking tests that we have done, we have caused angina occasionally. I think Davis has had that experience also. When one sees the ballistograph go to pieces after smoking a cigarette, one wonders whether it is safe to give nicotine to older patients. If the patient is smoking when trouble develops, the excess action stops quickly when he stops, but if an overdose is given by intravenous injection little can be done to hasten the end of the action.

Part VI. Other Aspects of the Pharmacology of Nicotine and Smoking

THE EFFECT ON RATS OF CHRONIC EXPOSURE TO CIGARETTE SMOKE*

H. B. Haag, P. S. Larson, J. H. Weatherby
Department of Pharmacology, Medical College of Virginia, Richmond, Va.

Prior to the time that the present studies on rats were undertaken, only a few observations had been reported on the effect on any animal species of chronic (longer than 6 months) exposure to cigarette smoke. These earlier studies dealt almost entirely with the effect on mice: for example, Mertens (1930, 1941) and Campbell (1936). Even at present, the only other extensive studies on rats, comparable to the ones to be reported are those by Passey and his colleagues (1956, 1957, 1958). These will be discussed below in relation to the results obtained in our experiments, in which rats were exposed 14 times a day to cigarette smoke for periods that, for some, embraced almost their entire life span.

Experimental

Smoking Machine and Exposure Chamber

Figure 1 represents a sectional view of the apparatus used for generating the cigarette smoke and the chamber in which the rats were exposed to the smoke. It was designed to simulate human smoking as closely as was practicable.

A synchronous clock motor, not shown, was equipped with contacts that actuated the puffing cycle at 1-min. intervals. Once started, control of the puffing cycle was transferred to a set of contacts on the cam shaft (A). When the cycle was completed, the contacts reset themselves, permitting the clock motor to start another cycle 1 min. later.

The actuating cam shaft (A) was driven through a set of reduction gears by a one-eighth horsepower induction motor and, once actuated, turned at a constant speed. Starting from the bottom position shown, with the slide valve (F) closed, the cam moved the diaphragm (D) through its connecting rod (C) and lever (B), raising the diaphragm (D) and thereby drawing air through the cigarettes (E).

A lighting device, consisting of a curved pipe carrying 12 small gas pilot flames, was swung into position manually for lighting the first puff of each of the 12 cigarettes used for every exposure.

At the top of the stroke, the slide valve (F) opened so that, on the downward stroke, the diaphragm (D) puffed smoke through the smoke pipe (G) and into the exposure chamber (K) through the smoke distributor (I). Five seconds later the centrifugal blower (H) started and effectively scavenged all smoke from the smoke pipe, blowing it through the distribution cylinder (I) that

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* A preliminary report on certain aspects of this study was presented before the 36th annual meeting of the American Society for Pharmacology and Experimental Therapeutics, at Atlantic City, N. J., March 11–15, 1946.[1]

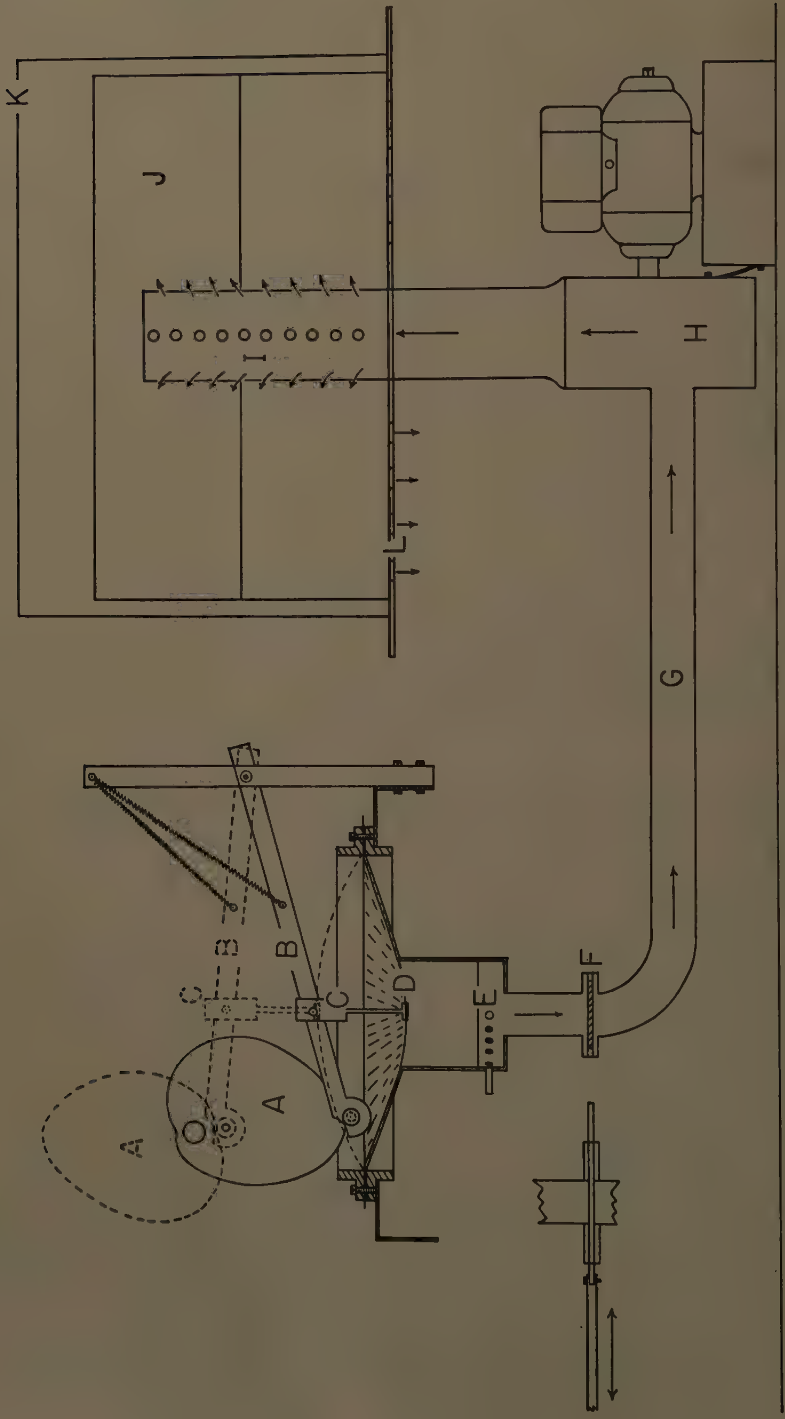

Figure 1. Sectional diagram of apparatus used for generating cigarette smoke and the chamber in which the rats were exposed to smoke.

extended vertically through the tier of two cages (J). These cages were circular, each containing eight individual compartments arranged around a hollow center that accommodated the smoke distribution cylinder. The cages were beneath a transparent hood (K) that rested upon a perforated base plate (L).

A small gap was left at the junction of the smoke pipe and blower intake that, by continued operation of the blower, permitted complete displacement of all smoke in the animal cage 40 seconds after the beginning of the puff. Since the puffing cycle was 2 seconds, and a 5-sec. interval occurred before the blower started, animals in the cage were exposed to smoke for approximately 33 seconds of each minute.

The volume of puffing was regulated by the size and shape of the actuating cam. As a check on design of the cam, the machine was connected to a spirometer and the volume of puffing checked by chart records. This was found to be 420 ml. per stroke, or the equivalent of 35 ml. per cigarette for each puff.

The volume of the exposure chamber and smoke pipe was 23 l. The dilution of each puff of smoke was of the order of 1:50; when the cages were occupied by rats this dilution would be about 1:40.

The cigarettes used were blends with an average weight of 1.09 gm. They contained 2.17 per cent nicotine and yielded, on an average, 10 puffs when smoked for two thirds of their length. The smoke from these 10 puffs contained, on an average, 3.3 mg. nicotine per cigarette. Nicotine analysis of the cigarette tobacco and the cigarette smoke was done according to the procedures used by Bradford *et al.* (1937).

Rats

Experiment 1. One lot of white rats, all about 38 days of age, was divided into 3 groups, each consisting of 12 females and four males. These groups contained equal numbers of litter-mates.

One group ("smoked") was exposed to approximately 10 puffs of cigarette smoke in the exposure chamber every half-hour 14 times daily for almost their entire life span. Another group ("smoke controls") was placed in a second apparatus but was exposed to puffs of air instead of smoke for the first 400 days of the experiment. From then to the end of the experiment, because this chamber was put to use for smoke exposure purposes, this group was kept during the smoking periods on a table near the rats being smoked and was thus exposed the the same general laboratory conditions. The third group ("cage controls") was kept continuously in the animal room.

All rats were deprived of food and water during the time the smoked animals were being exposed to smoke, except during the first eight months of the experiment when the cage controls had constant access to food and water. From 11:40 A.M. to 12:40 P.M. each day the smoked rats and the smoke controls were brought back to the animal room, and during this time all rats were allowed food (Purina Dog Chow) and water freely. At night all the rats were housed in the same animal room with food and water supplied ad libitum and, in addition, were offered slices of carrot.

The only time the regular schedule was interrupted was when blood pressure determinations were being made, when the smoking machine was being repaired or cleaned, and during mating, gestation, and lactation, when the females in all groups were kept continuously in the animal room. After mating was completed the routine was promptly reestablished for the males.

Food and water consumption was recorded daily. The rats were weighed once a week.

Experiment 2. This experiment, which was continued for two years, was undertaken to provide a larger number of observations on male rats. Thirty male rats of weaning age were divided into three equal groups and treated similarly to the rats in Experiment 1. There was no difference in the feeding period of the cage controls compared with the other two groups, as was the case in the early months of Experiment 1. The smoke controls in this experiment, unlike those in Experiment 1, were not at any time exposed to puffs of air.

In determining the effects of exposure to the cigarette smoke on the rats in both experiments, comparisons were made in terms of differences in weight, blood pressure, longevity, reproduction, and necropsy findings, between the exposed rats and those constituting the two control groups.

RESULTS

Effect of Smoke on Weight

Experiment 1. FIGURE 2 illustrates the weight changes, as averaged monthly, noted in the three groups of rats in Experiment 1. The female animals exposed to cigarette smoke achieved and maintained, initially, a slightly lower weight than those of either of the two control groups. The differences between the smoked rats and the cage controls were consistently statistically significant (P values of from <0.05 to >0.01) for about the first six months of the experiment. Thereafter there appeared to be no significant differences between these groups. Only in the very early stages of the experiment was there a significant difference between the smoked animals and the smoke controls or between the smoke controls and cage controls.

Gross inspection of the growth curves of the male animals in this experiment gives the impression that there was no difference between the smoked and the smoke control groups and that both of these groups achieved a smaller average weight than those of the cage controls. Because of the relatively few males in this experiment, statistical analysis for significance of difference was not made although this was done for the data of Experiment 2, which consisted entirely of males.

From the food consumption data it appears that the smaller weight of the smoked rats may have been associated with decreased food intake. However, because of uncontrollable food spillage, the reliability of the food consumption data is questionable. There seemed to be no difference in water consumption among any of the groups.

Experiment 2. The results obtained relating to weight changes in Experiment 2 (all males) are shown in FIGURE 3. In this experiment many of the same general relationships determined for Experiment 1 were found to exist. Throughout the experimental period there was a consistent and statistically significant difference between the smoked animals and the cage controls, whereas generally there was no such difference between the smoked rats and the smoke controls, and the smoke controls and the cage controls.

The apparent, although not statistically substantiated, difference between the weights of the smoke control group and the cage control animals may represent the influence of exposure to the general laboratory environment alone on growth, and suggests that the weight of the smoked rats reflects the composite effects of the general handling plus exposure to cigarette smoke.

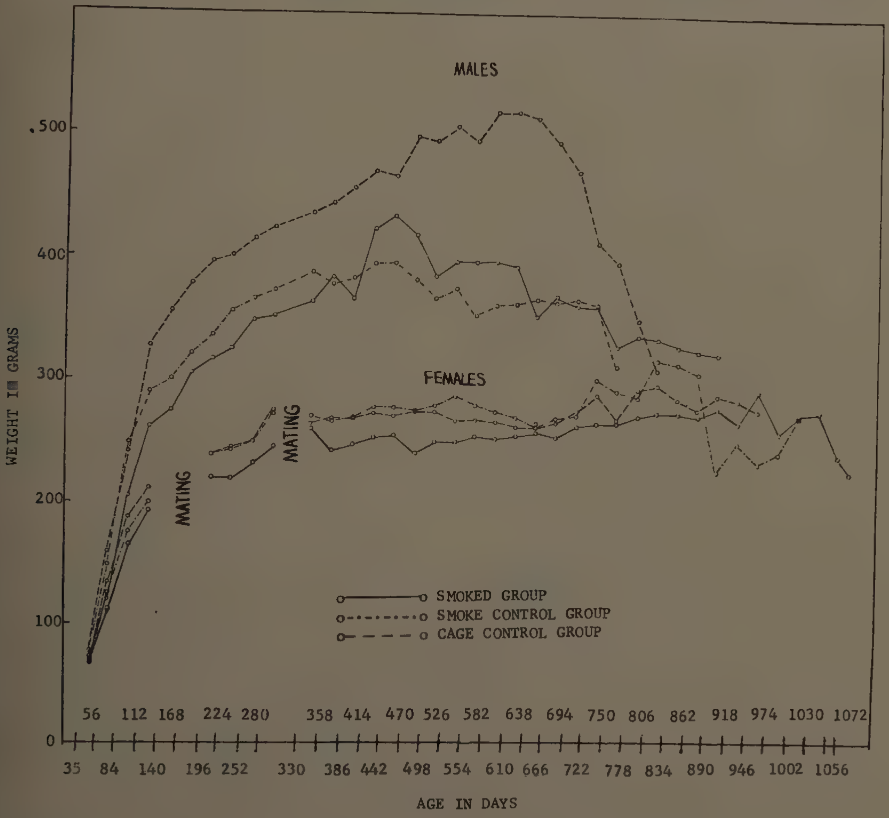

FIGURE 2. The effect of exposure to cigarette smoke on the growth of rats in Experiment 1.

In an attempt to determine the reversibility of effects of smoke exposure on weight, the smoked group and the smoke control group were interchanged when these rats were 478 days old. Thus the smoke controls were exposed to smoke, while the smoked rats became the smoke controls. As a result of this change the weight of the previous smoke control rats declined significantly to a level comparable to that of the original smoked group, while the previous smoked rats gained significantly in weight to reach the original level of the former smoke control animals. However, changing the new smoke controls finally into cage controls did not seem to lead to an enhancement of their growth to equal that of the original cage controls. Again, converting the

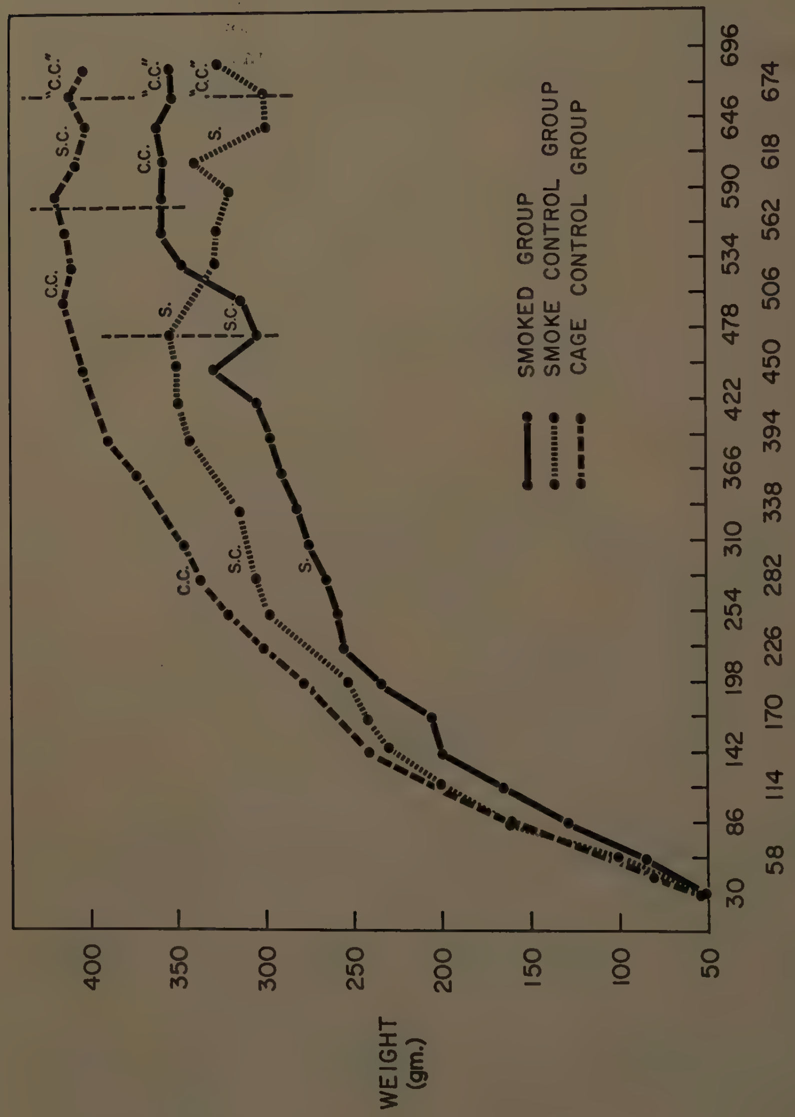

FIGURE 3. The effect of exposure to cigarette smoke on the growth of rats in Experiment 2 (all males).

original cage controls to smoke controls produced only a very slight diminution in their weight. Finally, when the new smoked group was changed into cage controls their weight showed a quick upturn towards their initial level.

Effect of Cigarette Smoke on Blood Pressure

Blood pressure was measured by the method of Williams *et al.* (1939).

During the first two years of Experiment 1 blood pressure determinations were made at monthly intervals on the three groups of rats, about 12 hours after the last smoke exposure. FIGURE 4 illustrates the average values obtained. It is apparent that exposure to cigarette smoke did not produce chronic hypertension. As a matter of fact, gross inspection of the three blood pressure curves may give the impression that the smoked rats on aging (24 months and beyond) had a lower pressure than either the smoke controls or the cage controls. However, statistical analysis showed no significant difference to exist.

When blood pressure was determined immediately prior to and after exposure to cigarette smoke, a slight though statistically significant (P = 0.01) increase (from 103.6 mm. Hg before exposure to 108.5 after) was noted in a group of nine smoked animals 611 days old. This effect persisted, on an average, for 45 minutes. Duplication of this experiment three weeks later gave similar values.

Effect of Cigarette Smoke on Longevity

In Experiment 1 all of the cage control rats had died within 31 months, the smoke control ones within 33 months, and the smoked animals within 35 months. The average life span of the smoked rats was 727 ± 251 days, of the smoke controls 617 ± 271, and of the cage controls 714 ± 141 days. The differences between these figures are not statistically significant. The longest-lived animal was a female in the smoked group that lived to be 1076 days old.

In Experiment 2 three fatalities had occurred in the smoked group, and five each in the smoke control and cage control groups when the experiment was terminated at the end of 2 years. There was no statistically significant difference between the average ages of these rats at death.

The Effect of Cigarette Smoke on Reproduction

When the rats in Experiment 1 were 120 days old, 10 females in each group were served by 3 males of the same group, for a period of 14 days. A similar experiment was done when the rats were 240 days old. Because of technical difficulties in arranging what might be considered adequate control conditions, none of the females were subjected to cigarette smoke during mating, gestation, and lactation; all rats were kept under identical conditions in the animal room during this period. After the mating interval of two weeks the males were again treated as described above.

The results of observations made of the incidence of pregnancy, litter size, mortality, still births, cannibalism, and maternal neglect are summarized in TABLE 1. There were no apparent differences between the results noted in the smoked group and the two control groups.

All surviving offspring were sacrificed at 90 days and observations made of

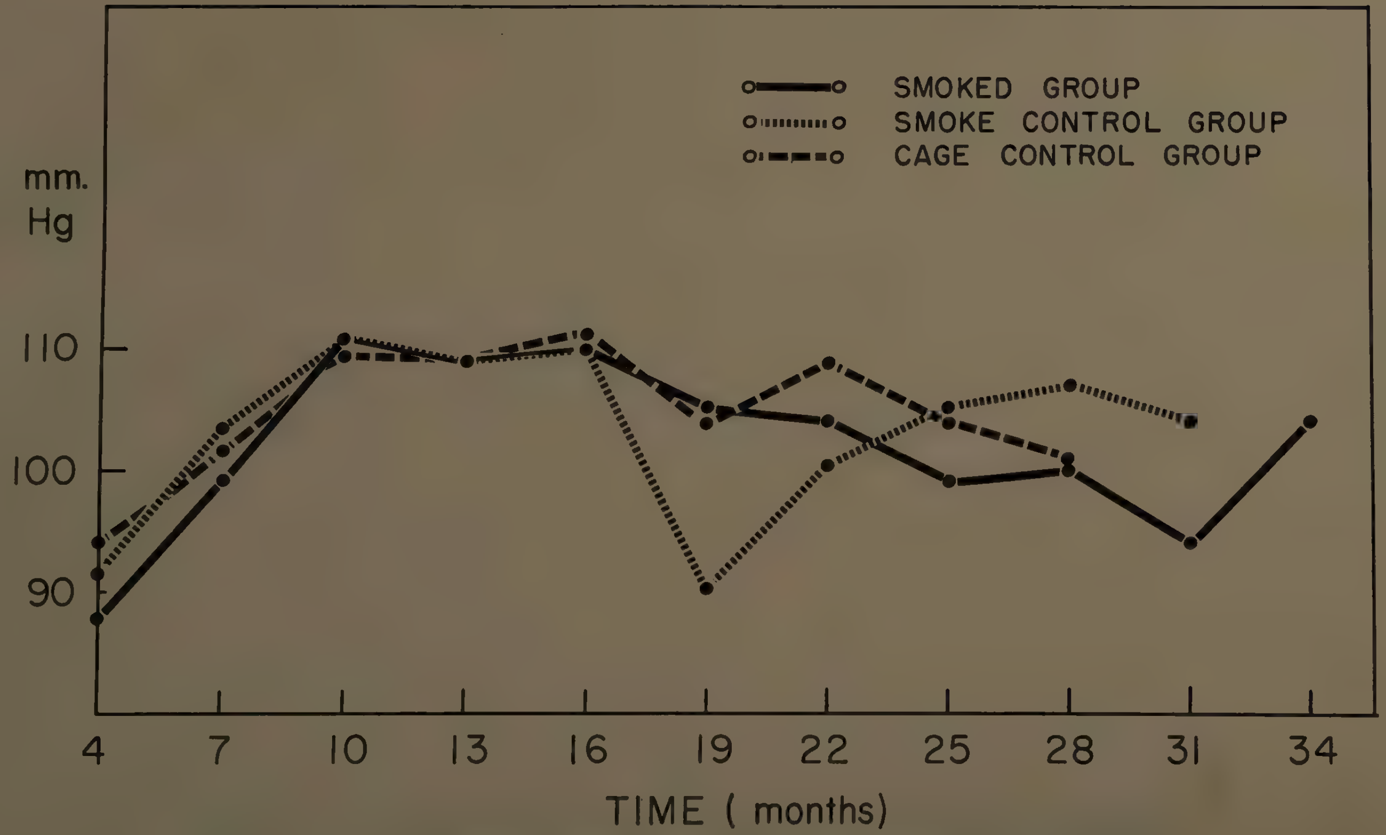

FIGURE 4. The effect of exposure to cigarette smoke on the blood pressure of rats in Experiment 1.

body length, body and representative organ weights, and red and white cell counts. These observations are summarized in TABLE 2. Again no differences between the offspring of the exposed group and the control groups were apparent.

TABLE 1

THE EFFECT OF EXPOSURE TO CIGARETTE SMOKE ON NUMBER OF LITTERS, LITTER SIZE, AND MORTALITY OF RATS IN EXPERIMENT 1

	First mating (120 days)			Second mating (270 days)		
	Smoked	Smoke controls	Cage controls	Smoked	Smoke controls	Cage controls
No. females served	10	10	10	10	9	10
No. litters	7	7	8	6	8	8
Av. litter size	9.4	7.7	9.4	7.2	9	9.3
Per cent mortality	56.1	61.1	3.6	11.6	36.1	18.9
Per cent deaths due to still birth	1.5	1.9	2.7	7	4.2	17.6*
Per cent deaths due to cannibalism	4.6	13	9.3	4.6	8.3	1.3
Per cent deaths due to neglect	50	46.2	24	0	23.6	0

* One entire litter stillborn.

TABLE 2

BODY LENGTHS, BODY AND ORGAN WEIGHTS, AND RED AND WHITE CELL COUNTS OF 90-DAY OFFSPRING OF SMOKED AND CONTROL RATS IN EXPERIMENT 1

Average values	Sex	First-born			Second-born		
		Smoked	Smoke controls	Cage controls	Smoked	Smoke controls	Cage controls
Body length (cm.)	F	17.5	17.2	17.6	18.3	18.4	18.0
	M	18.8	18.4	17.1	18.6	19.0	19.2
Body weight (gm.)	F	148	140	130	154	153	143
	M	189	167	147	172	177	184
Heart (gm.)	F	0.62	0.61	0.51	0.65	0.64	0.59
	M	0.75	0.64	0.55	0.65	0.69	0.69
Kidney (gm.)	F	1.2	1.2	1.1	1.2	1.3	1.1
	M	1.5	1.3	1.1	1.4	1.4	1.5
Liver (gm.)	F	6.8	7.3	6.0	6.5	7.1	6.2
	M	8.6	7.9	6.9	7.4	7.9	8.2
Spleen (gm.)	F	0.39	0.33	0.41	0.39	0.33	0.42
	M	0.46	0.38	0.38	0.39	0.36	0.46
Adrenals (gm.)	F	0.038	0.038	0.031	0.034	0.035	0.033
	M	0.028	0.026	0.024	0.021	0.020	0.022
Gonads (gm.)	F	0.062	0.074	0.056	0.076	0.088	0.061
	M	2.1	2.2	1.8	2.0	2.2	2.2
White cells × 1000	F	4.5	4.3	4.4	6.5	6.7	6.9
	M	4.9	4.6	4.4	7.1	6.8	7.1
Red cells × 1,000,000	F	8.9	8.4	7.8	9.1	8.9	8.7
	M	8.9	8.7	7.8	8.8	8.9	8.8

In addition, histopathological studies were made on 11 males and 12 females from the first litters and 19 males and 18 females from the second litters. In the case of the first litters these embraced two sibling pairs from each of two litters in all three groups, excepting the smoke control group, in which a total of three males was taken from two litters. In the case of the second litters these embraced sibling pairs from each litter excepting the smoked group, from which 11 animals were selected from 6 litters. No pathologic features characteristic of any of the groups were revealed.

TABLE 3
ABNORMAL AUTOPSY FINDINGS IN RATS OF EXPERIMENTS 1 AND 2

Lesions	Smoked rats			Smoke control rats			Cage control rats		
	Exp. 1 (15/16)*	Exp. 2 (10/10)	Totals	Exp. 1 (12/16)	Exp. 2 (10/10)	Totals	Exp. 1 (14/16)	Exp. 2 (9/10)	Totals
Lungs									
(a) Pneumonia	—	2	2	—	1	1	2	1	3
(b) Bronchiectasis	12	6	18	10†	7	17	11	5‡	16
(c) Tumors, malignant	—	—	—	1†	—	1	—	3‡	3
Heart									
(a) Interstitial myocarditis	1	1	2	1	—	1	—	1	1
(b) Suppurative pericarditis	—	—	—	—	—	—	1	—	1
(c) Chronic pericarditis	—	1	1	—	—	—	—	—	—
Liver									
(a) Acute focal hepatitis	2	—	2	1	—	1	2	—	2
(b) Focal necrosis	1	2	3	—	—	—	—	—	—
(c) Granuloma	—	—	—	2	—	2	—	—	—
Kidney									
(a) Interstitial nephritis	—	1	1	2	3	5	—	1	1
(b) Glomerulonephritis	—	3	3	—	—	—	—	2	2
(c) Hydronephrosis	1	—	1	—	—	—	—	—	—
Tumors									
I. Benign									
(a) Fibroadenoma of breast	3	—	3	2	—	2	1	—	1
(b) Fibroadenoma of pelvis	1	—	1	—	—	—	—	—	—
(c) Adrenal adenoma	—	—	—	—	—	—	1	—	1
II. Malignant									
(a) Lymphosarcoma									
(1) Ileocecal	3	1	4	2	—	2	1	1	2
(2) Pulmonary	—	—	—	—	—	—	—	3	3
(3) Mediastinal (thymus)	1	—	1	—	—	—	—	—	—
(b) Fibrosarcoma of breast	1	—	1	1	—	1	—	—	—
(c) Carcinoma									
(1) Liver	1	—	1	—	—	—	—	—	—
(2) Sigmoid colon	—	—	—	—	—	—	1	—	1
(3) Lung	—	—	—	1	—	1	—	—	—
(4) Uterus	1	—	1	—	—	—	—	—	—
(5) Face	—	—	—	1	—	1	—	—	—

* Number of rats autopsied over number of rats in experiment.
† Rat No. 26 showed both bronchiectasis and carcinoma of lung.
‡ Rat No. 72 showed both bronchiectasis and lymphosarcoma of lung.

Effect of Cigarette Smoke on Pathological Anatomical Changes

Histopathological examination was made on 70 of the 78 animals in Experiments 1 and 2; 25 from the smoked groups (15 from Experiment 1, 10 from Experiment 2), 22 from the smoke control groups (12 from Experiment 1 and

10 from Experiment 2), and 23 from the cage control groups (14 from Experiment 1 and 9 from Experiment 2). Lesions encountered and their frequency are summarized in TABLE 3 and lead to the conclusion that there were no lesions peculiar to the rats exposed to smoke.

DISCUSSION

Our observation that the rats exposed to cigarette smoke achieved and maintained a lower weight than did the control animals is in keeping with the observations of Passey (1957, 1958). Whereas this might be related to a diminished food intake that our food consumption figures, as well as those of Passey, indicated had occurred, Passey felt that in the smoked rat there might also be a change in the manner of food utilization.

Passey (1958) reported that his failure during a five-year period of experimentation to induce lung tumors in rats by exposure to strong concentrations of cigarette smoke is a strikingly negative result. The only lung cancer he found was a solitary tumor in a control rat, and this was associated with marked bronchiectasis. Similarly, the only malignant pulmonary tumors encountered in our experiments also occurred only in control animals.

It is interesting to note that Passey *et al.* (1956) remarked on the presence of bronchiectasis in their rats. In our own experiments we found a very high and almost equal incidence of bronchiectasis in all of the groups of rats, smoked and controls alike, thus making it evident that this condition was related to the aging process rather than to the exposure to cigarette smoke.

It would appear pertinent to make one general observation regarding the problem of studying the effects on animals of exposure to cigarette smoke. There is a very apparent need for writers in this field to describe in sufficient detail the materials and techniques employed in their work to allow reasonable comparisons with other animal studies of similar nature, and with the conditions existing in human smoking. To provide a standard of reference, there should be general agreement on methods of generating the cigarette smoke, size of exposure chamber, and number and length of daily exposures.

SUMMARY

One group of 12 female and 4 male rats was exposed to cigarette smoke 14 times daily for almost their entire life spans, and another group of 10 males was similarly treated for 2 years. In comparison with control groups it was found that rats exposed to cigarette smoke achieved and maintained a slightly lower weight, and showed no significant differences in blood pressure, longevity, reproduction, and observations at autopsy.

ACKNOWLEDGMENTS

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REFERENCES

BRADFORD, J. A., E. S. HARLOW, W. R. HARLAN & H. R. HANMER. 1937. Nature of cigaret smoke-volatile bases and acids. Ind. Eng. Chem. **29:** 45–50.

CAMPBELL, J. A. 1936. The effects of exhaust gases from internal combustion engines and of tobacco smoke upon mice, with special reference to incidence of tumors of the lung. Brit. J. Exptl. Pathol. **17:** 146–158.

Mertens, V. E. 1930. Zigarettenrauch eine Ursache des Lungenkrebs. Z. Krebsforsch. **32:** 82–91.

Mertens, V. E. 1941. Noch einmal Zigarettenrauch und Lungenkrebs. Z. Krebsforsch. **51:** 183–192.

Passey, R. D., E. Boyland, B. M. G. Pratt & I. Hieger. 1956. Cigarette smoking and lung cancer. Brit. Emp. Cancer Campaign Ann. Rept. **34:** 15–16.

Passey, R. D. 1957. Carcinogenicity of cigarette tars. Brit. Emp. Cancer Campaign Ann. Rept. **35:** 65–66.

Passey, R. D. 1958. Cigarette smoke and lung cancer. Brit. Emp. Cancer Campaign Ann. Rept. **36:** 48–49.

Williams, J. R., Jr., T. R. Harrison & A. Grollman. 1939. A simple method for determining the systolic blood pressure of the unanesthetized rat. J. Clin. Invest. **18:** 373–376.

1. Federation Proceedings. 1946. **5:** 181–182.

CHRONIC NICOTINE POISONING

Clinton H. Thienes
Institute of Medical Research, Huntington Memorial Hospital, Pasadena, Calif.

The older literature contains many reports on the deleterious effects of chronic nicotine poisoning, describing retardation of growth,[1-5] tolerance development,[6-12] interference with reproduction,[2,13,14] enlargement or injury of the adrenals,[2,15-20] atrophy of the gonads,[2,17,21] changes in other endocrine organs,[17,22] and production of arterial disease.[23-32] Because most of the reports were based upon poorly controlled experiments or on too small a number of animals, my associates and I initiated a systematic reinvestigation of the chronic effects of nicotine, employing littermate or other closely related controls. Test animals received subconvulsive doses of nicotine dissolved in 0.9 per cent saline, and controls were injected with the same volumes of saline. Experiments extended over intervals of three months to a year. Thus was demonstrated a species difference with respect to growth,[33,34] a decrease in fertility of female rats but not of male rats,[35] but without gross or histological evidence of organ injury, and the development of drug tolerance of the chronically poisoned animals.[36]

Methods

Merck's nicotine was used in all experiments, and was water-clear when purchased. Toward the end of a year's experiment the nicotine would become tinged with yellow. The 1 per cent stock solution of nicotine in 0.9 per cent saline was made fresh every second week and the final dilutions were made daily. The drug was injected subcutaneously at 8:00 and 4:30 P.M., Monday through Friday, and at 9:00 A.M. Saturday morning; with 10-day-old rats and mice the dose was 1 to 2 mg./kg. as a 0.1 per cent solution twice daily, and this dose was gradually increased over a period of 2 weeks to a month until 5 mg./kg. was being given. As the animals grew the concentration was increased so that the total volume injected did not exceed 1 ml. Injections were started when the animals were about 10 days old, that is, when there was sufficient hair to permit marking with picric acid solution for identification. As nearly equally as possible, male and female young were divided into test and control groups, and neck, tail, or back region was marked with picric acid solution.

The young animals were left with their mothers until four weeks old. The young mice were then placed in cages in groups of about twenty animals, half control and half nicotine-injected. The bedding was excelsior. In the case of rats a male and a female were placed together in a cage compartment, with a control pair on one side of a hardware cloth divider and a nicotine-injected pair on the other side. In the first experiment the rats rested on a one-half-inch mesh hardware cloth floor, but since even with nesting boxes baby rats would occasionally fall through the mesh, subsequent experiments were carried out with the rats resting on fir shavings in the pan at the bottom of the cage. In the first two experiments, the food was Purina Lab Chow; in the later experiments Gaines Chunks was fed. The food was weighed during two of the experiments.

The animals were weighed each day before injection until they weighed approximately 50 gm., after which they were weighed only once per week. The dose of nicotine was given according to the weight record, and according to the symptoms produced in individual animals. If a convulsion occurred twice within a week, the dose would be decreased by one half, and then gradually increased to the standard dose. The young were counted and weighed on day of birth; males and females were recorded separately. A similar record was made on the day of weaning.

Outdoor temperature prevailed in the first two experiments, but subsequently the animal quarters were kept under thermostatic control.

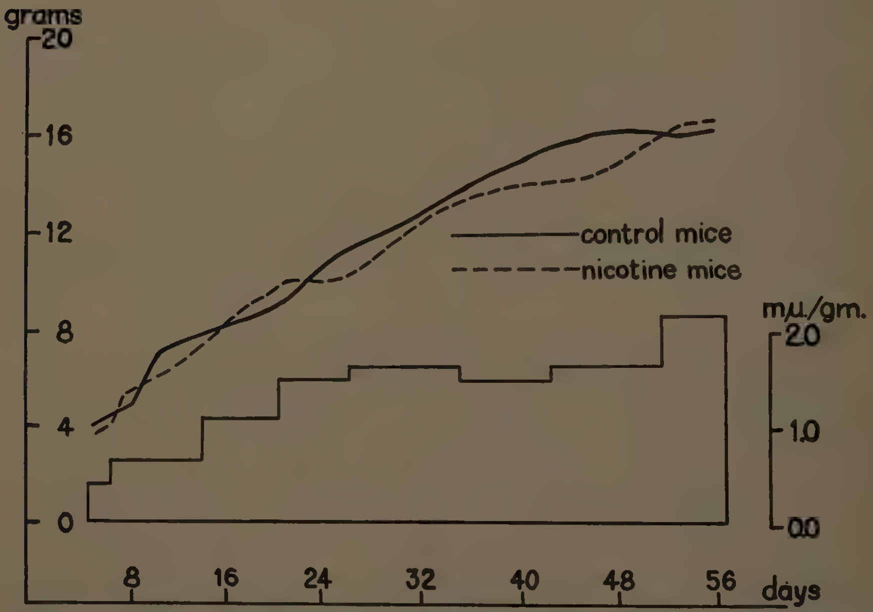

FIGURE 1. Growth curves of control and nicotine-injected mice. The dose of nicotine is shown in the histogram. Reprinted by permission of *The American Journal of Hygiene*.[33]

Results

Growth. The growth rate of mice, indicated by weight change, did not seem to be influenced by nicotine. FIGURE 1 represents the weight increase of the groups of mice. In the case of rats, however, the weight-gain of the nicotine-injected males was significantly less than that of the controls, and toward the end of the experiment the average difference in weights was about 12 per cent. The difference between the test and control female rats was not as great, and at times was not statistically significant, although at all points along the curve, the average weight of the test females was less than of the controls. FIGURES 2 and 3 show the average growth rates.

When tip-of-nose to base-of-tail measurements of the two groups of rats were compared, no difference could be found. This confirmed the results of Fields, who measured the femur lengths.[37]

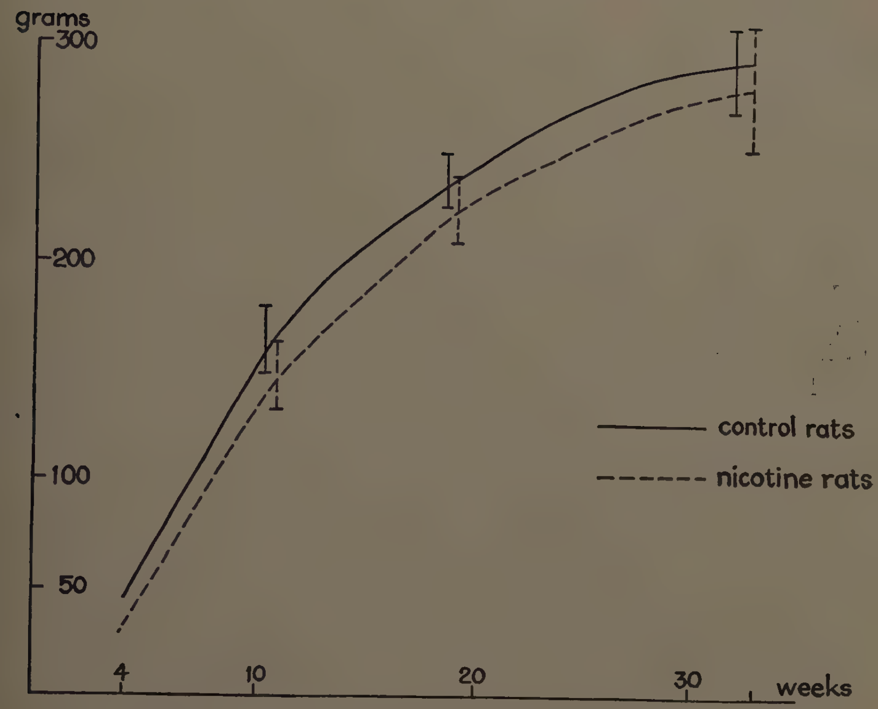

FIGURE 2. Growth curves of male rats. The vertical lines represent standard deviation.

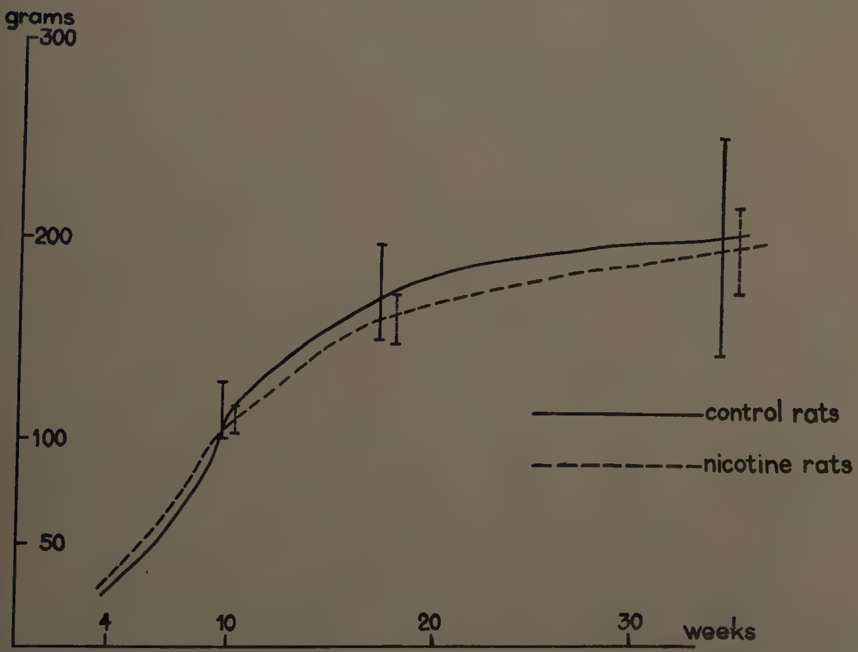

FIGURE 3. Growth curves of female rats. The vertical lines represent standard deviation.

When the abdomens of the rats were opened, the difference in amounts of fat was readily noticed. Hence the intra-abdominal fat was removed and weighed, and was found to be less than one half as much in the nicotine-injected rats as in the controls. Since there was no difference in body lengths of the two groups of rats, it was concluded that the difference in weights of the animals was due to the marked decrease in body fat in the nicotinized rats. This was confirmed by Smith *et al.*,[38] who measured total body fat.

Tolerance development. Tolerance to small doses was measured by determining the smallest dose that would cause weakness of the hindquarters and cerebral excitation, resulting in the animal resting on its gluteal region while its forelegs pivoted the body. A dose was considered effective when the body was pivoted through a complete circle three times or more. This is designated the pivoting response. Determinations of tolerance were made about once per month. A second or a third test was sometimes necessary to determine degree of tolerance, in which case no more than two test injections were given in any one week, and the usual morning dose was omitted on the day of the test.

Within two weeks a measurable tolerance to small doses of nicotine had developed in rats and this increased to a four- to ninefold tolerance at six months, as measured by the pivoting response. There seemed to be no tolerance to convulsive or fatal doses. Tolerance development was greatest when injections were begun at an early age; 1-year-old rats developed at best a 30 per cent tolerance to the small doses, and many developed no tolerance (FIGURE 4).

A few preliminary experiments indicated the development of tolerance of the adrenal medulla to the stimulating effect of nicotine. The animals were anesthetized with pentobarbital and an adrenal gland was removed. An injection of either nicotine or saline alone was then given and one half hour later the other adrenal was removed. Biological assay for active catecholamines on rabbit intestine showed (1) nearly twice as much potency of the extracts of resting glands from the test animals as from controls[39] and (2) less loss of potency of the adrenal extracts due to the nicotine injection in chronically poisoned animals than in control animals. Injection of saline caused no loss of catecholamines. This type of tolerance requires further study.

Reproduction. Three similar experiments were conducted, each approximately 1 year long, with 25 to 50 pairs of rats in control and nicotine groups. In a fourth experiment only females of one group and only males of a second group received nicotine; in the negative control group, both male and female received injections of saline.

Sterility. The number of sterile matings was twice as great among nicotine-injected pairs and among pairs in which only the female received nicotine than among control pairs or pairs in which only the male received nicotine. A pair of rats was considered to be sterile if the female bore no young during the year of the experiment.

Progeny. The number of litters of young born to fertile pairs of rats and the number of total young born was about two thirds as great among the nicotine-poisoned rats as among the controls. Since some of the matings resulted in no births, the calculations were based only on the number of young born to fertile pairs and, in order to make the comparisons more exact, the

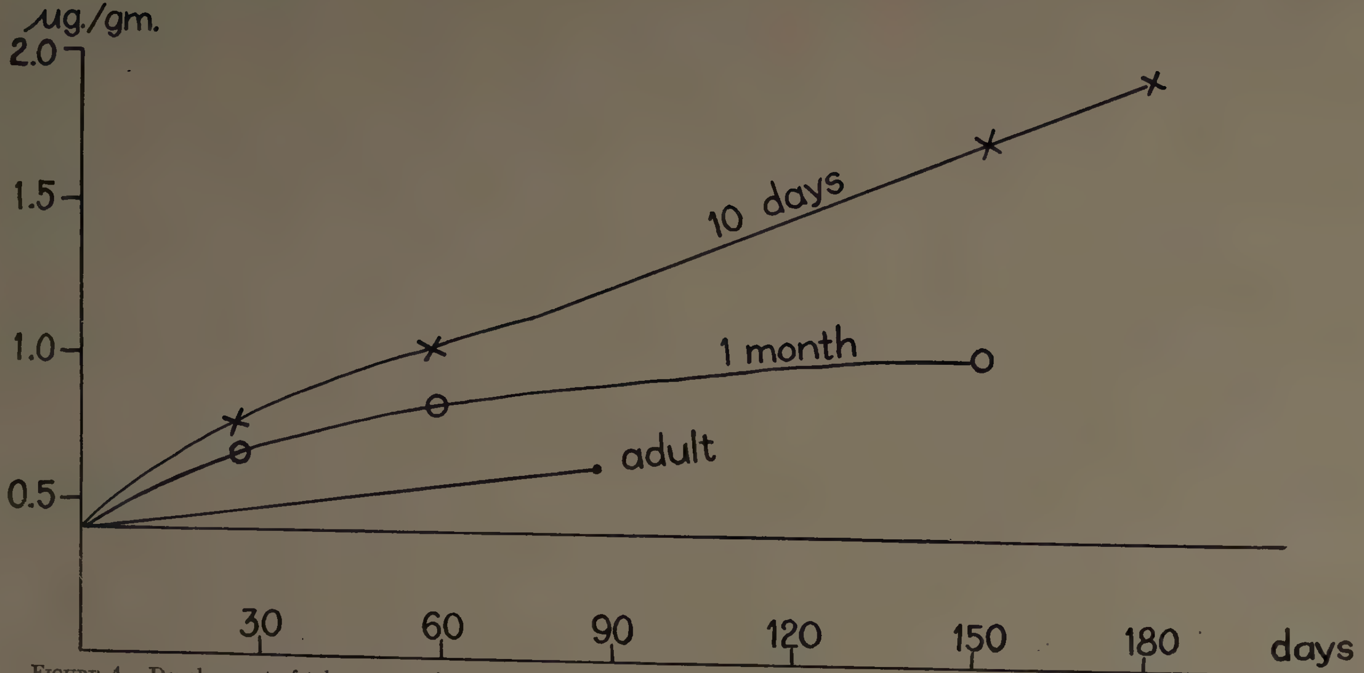

Figure 4. Development of tolerance to nicotine. The degree of tolerance development is an inverse function of the age at which administration of nicotine was started.

factor of "mating days" was used in determining the litter index and the progeny index. Mating days was defined as the sum of the number of days that each pair of rats in a group received injections, and the litter and progeny indices are the number of litters and number of total young for each group divided by the mating days of each respective group. From TABLES 1 and 2 it may be seen that there were only 75.5 per cent as many litters and 77.9 per cent as many young born to nicotine poisoned fertile rats as to the controls when calculated on the basis of mating days. Had both fertile and nonfertile rats entered into the calculations, the percentages would have been smaller.

Geller[40] failed to confirm our results on rats, but Simpson[41] and Lowe[42] both reported that the birth weights of human infants from smoking mothers were definitely lower than from nonsmoking mothers. Simpson interpreted her results to mean that smoking mothers had twice as many premature babies as nonsmoking mothers, but Lowe could find no evidence for this. Simpson considered all babies under five pounds as premature. We found no difference in birth weights of rats in our two groups of rats.

TABLE 1
EFFECTS OF CHRONIC NICOTINISM ON REPRODUCTION IN RATS

	Nonfertile pairs (%)	No. litters from fertile pairs	Mating days	Litter index
Controls				
92 pairs total	18.75			
78 pairs fertile		287	21,223	0.0135
Nicotine-treated				
82 pairs total	32.35			
61 pairs fertile		162	15,832	0.0102

TABLE 2
EFFECTS OF CHRONIC NICOTINISM ON REPRODUCTION IN FERTILE RATS

	No. progeny	Mating days	Progeny index
Controls			
78 pairs	1883	21,223	0.0885
Nicotine-treated			
61 pairs	1093	15,831	0.0690

Earlier studies on effects of smoking on childbearing are fragmentary. Chiasson[43] reported no effect (or beneficial effect) among settlers in Nova Scotia, as opposed to results reported in "A Foreign Letter" from Germany in *The Journal of the American Medical Association*,[44] which reported a high incidence of miscarriages in women working in the tobacco industry. Phillips[45] observed disappearance of motile sperm from the semen of a male patient during smoking and return of motile sperm on stopping smoking. This is contrary to our results, which showed that chronic nicotinism decreases fertility of female rats but not of male rats.

Mortality of young. The number of young that lived until weaning was determined for one experiment and summarized in TABLE 3. With these figures taken into consideration along with the number of nonfertile pairs, it is apparent that but little more than half as many four-week-old young were produced by the nicotine-poisoned animals as by the controls. The mortality rate was the same for males as for females.

Essenberg[13] confirmed our results in general. A new type of observation reported by Nishimura and Nakai,[46] but one that needs confirmation, is that anomalies occur among offspring of mice chronically poisoned with nicotine.

Adrenal size. At the end of a one-year-long experiment, 50 control and 50 nicotine-treated rats were decapitated and the adrenal glands were quickly removed, cleared of surrounding fat and extraneous tissue, weighed on a torsion balance, and placed in 4 per cent formaldehyde (10 per cent formalin). Later the hardened glands were imbedded in paraffin, sectioned and stained. Every fifth section was drawn on paper with the aid of camera lucida, outlining both the medulla and cortex, which were measured by planimetry; from these measurements the total volumes of the medulla and cortex could be calculated.

The ratio of adrenal weight to body weight and to body area was increased 10 to 15 per cent by nicotine. However, when total volumes of the adrenals from control and nicotine-treated were compared it was seen that there was no difference; neither was there any difference in the ratios of cortex to medulla in the two groups of glands. Hence the difference of adrenal weight to body-weight ratios must have been due to the decrease in body fat in the nicotine-injected rats.

TABLE 3

EFFECT OF CHRONIC NICOTINE POISONING ON MORTALITY OF YOUNG RATS

Parents*	No. born	No. weaned	Per cent mortality
N♀ C♂	550	213	62
C♀ N♂	481	288	41
C♀ C♂	527	312	42

* N = nicotine-injected. C = control (saline-injected).

A recent paper by Eränko *et al.*[47] reports that administration of nicotine for from 6 to 9 months caused a 110 per cent increase in the size of the adrenal medulla of rats, but not of mice or guinea pigs. Since the dose was similar to that administered in my experiments, but given only once daily, the difference from my results cannot be explained, except perhaps on the basis of the small number of animals (5 to 7 per group) used by Eränko and his co-workers, preventing a true random sample from being obtained. It is interesting that these investigators demonstrated a large increase in norepinephrine but not of epinephrine. My associates and I found twice as much potency of adrenal extracts from nicotine-poisoned rats as from controls, and designated the active material as epinephrine.[39] At the time of the report in 1941 the presence of norepinephrine in adrenal extracts was not suspected.

Pituitary and sex glands. When change in body weight of nicotine-injected rats is considered, no change in weights of pituitary or sex glands could be noted. Nor was there obvious change in their microscopic appearance when stained with hematoxylin and eosin.

Using tobacco smoke, Essenberg[48] caused damage to ovaries and testes, but no visible change in the pituitaries of mice. There was also a decrease in number of young born and carried through the nursing period.

Blood vessels. Coronary arteries and portions of the ascending and renal artery regions of the aortas of male control and chronically poisoned rats were examined grossly and microscopically with the collaboration of E. M. Butt, pathologist.[49] The animals had been injected with nicotine twice daily for a year and were slightly more than one year old at time of autopsy. No pathology attributable to the nicotine injections could be found. Medial degeneration was found more frequently in the controls than in the poisoned animals. Perhaps if the experiments had been performed in older animals, different results would have been obtained, but there is as yet no experimental or clinical evidence that nicotine produces arterial pathology. The increased mortality from coronary heart disease among smokers[50-53] may be on a different basis.

Summary

(1) Subconvulsive doses of nicotine, given twice daily for from 8 to 12 months to rats, results in decreased weight gain, due to decreased fat deposits. Proportionate doses given to white mice for 2 months had no effect on growth.

(2) Similar doses were associated with a decreased number of fertile matings, a decrease in number of litters and total young born, and an increase in mortality of the young during the nursing period.

(3) There was marked development of tolerance to small doses, causing hind-leg weakness when injections were given to young animals, but little or no tolerance to convulsive or fatal doses. When nicotine administration was begun after the adult size had been attained, only slight tolerance developed. There was also evidence of tolerance of the adrenal gland to nicotine, and the adrenal glands acquired an increase in sympathomimetic potency.

(4) There was no increase in size of endocrine glands in nicotinized rats.

(5) Chronic nicotinism did not cause vascular pathology.

References

1. Richon, L. & M. Perrin. 1908. Retards de dévelopment par intoxication tabagique expérimentale, possibilité de la reprisse de la croissance après cessation de l'intoxication. Compt. rend. soc. biol. **64:** 563.
2. Nakazawa, R. 1931. Der Einflusz der chronischen Nicotinvergiftung auf die Geschlechtsfunktion der weiblichen Ratten. Japan. J. Med. Sci. Pharmacol. **5:** 109.
3. Wilson, R. H. & F. De Eds. 1936. Chronic nicotine toxicity: I. Feeding of nicotine sulfate, tannate, and bentonite. J. Ind. Hyg. Toxicol. **18:** 553.
4. Pechstein, L. A. & W. R. Reynolds. 1937. The effect of tobacco smoke on the growth and learning behavior of the albino rat and its progeny. J. Comp. Psychol. **24:** 459.
5. Haag, H. B., J. H. Weatherby, D. Fordham & P. S. Larson. 1946. The effect on rats of daily life span exposure to cigarette smoke. Federation Proc. **5:** 181.
6. Hatcher, R. A. 1904. Nicotine tolerance in rabbits, and the difference in the fatal dose in adult and young guinea pigs. Am. J. Physiol. **11:** 1.
7. Edmunds, C. W. 1909. Studies in tolerance. I. Nicotine and Lobeline. J. Pharmacol. Exptl. Therap. **1:** 27.
8. Dixon, W. E. & W. E. Lee. 1912. Tolerance to nicotine. Quart. J. Exptl. Physiol. **5:** 373.
9. Diller, T. 1929. Tobacco smoking. J. Am. Med. Assoc. **93:** 939.
10. Binet, L. & C. Zamfir. 1931. Recherches expérimentales sur l'intoxication par le tabac et par le nicotine. Bull. mém. soc. méd. hôp. Paris. **47:** 1106.

11. Winsor, A. L. & S. J. Richards. 1935. Development of tolerance for cigarettes. J. Exptl. Psychol. **18:** 113.

12. Okomura, Y. 1937. Tolerance to nicotine in different species. Jikken Yakubutsugaku Zasshi. **13:** 277.

13. Essenberg, J. M., J. V. Schwind & A. R. Patras. 1940. The effects of nicotine and cigaret smoke on pregnant female albino rats and their offspring. J. Lab. Clin. Med. **25:** 708.

14. Willson, J. R. 1942. The effect of nicotine on lactation in white mice. Am. J. Obstet. Gynecol. **43:** 839.

15. Kosdoba, A. S. 1929. Zur Frage der experimentellen Pathologie der Nebennieren bei intravenöser Nikotineinverleibung. Arch. klin. Chir. **156:** 550.

16*a*. Staemmler, M. 1932. Nikotin und Nebennieren. Deut. med. Wochschr. **58:** 1960.

16*b*. Staemmler, M. 1935. Die chronische Vergiftung mit Nikotin. Arch. pathol. Anat. u. Physiol. Virchow's. **295:** 366.

16*c*. Staemmler, M. 1936. Über Geswulstartige Bildungen im Nebennierenmark als Folge experimenteller Nikotinvergiftung. Klin. Woch. **15:** 404.

17. Stadlander, K. H. 1936. Effect of nicotine on gonads, suprarenals and thyroid. Z. ges. exptl. Med. **99:** 670.

18. Kin, S. S. 1937. The influence of continuous injections of nicotine on the adrenal gland and the anterior lobe of the hypophysis. Japan. J. Med. Sci. Pharmacol. **10:** 59.

19. Kobayashi, S. 1937. Influence of nicotine on the adrenaline of the suprarenals in rabbits. Chem. Abstr. **31:** 6734.

20. Mosinger, M. & P. Bonifaci. 1939. Sur le réaction histophysiologique des glandes endocrines chez le cobaye soumis à la nicotinisation chronique. Compt. rend. soc. biol. **130:** 1292.

21. Wilson, R. H., J. B. McNaught & F. De Eds. 1938. Chronic nicotine toxicity. IV. Effect of nicotine-containing diets on histology and weights of ovaries of albino rats. J. Ind. Hyg. **20:** 468.

22. Erbacher, K., P. Grundbrecht & A. Loeser. 1940. Nikotin und innere Sekretion. I. Funktionstörungen innersekretorischer organe durch Nikotin. Arch. exptl. Pathol. Pharmakol Naunyn-Schmiedeberg's. **195:** 121.

23. Adler, I. & O. Hensel. 1906. Intravenous injections of nicotine and their effects upon the aorta of rabbits. J. Med. Research. **15:** 229.

24. Bayloc, M. J. 1906. Athérome expérimental de l'aorte consecutif à l'action du tabac. Compt. rend. soc. biol. **60:** 935.

25. Gouget, A. 1906. Sur quelques lesions de l'intoxication tabagique expérimentale. Presse méd. **14:** 533.

26. Lesieur, C. 1907. Tabagisme expérimental et denicotinisation. Compt. rend. soc. biol. **62:** 430.

27. Zebrowski, E. 1907. Zur Frage über die wirkung des Tabakrauches auf die Blutgefässe bei Thiere. Centrl. allgem. Pathol. u. pathol. Anat. **18:** 337.

28. Guillan, G. & A. Gy. 1908. Les lésions hepatique dans l'intoxication tabagique expérimentale. Compt. rend. soc. biol. **65:** 482.

29. Lee, W. E. 1908. The action of tobacco smoke with special reference to arterial pressure and degeneration. Quart. J. Physiol. **1:** 335.

30. Romm, S. O. & A. S. Kuschnir. 1928. Funktionelle Veränderungen der Herz- und Nierengefässe bei chronischer Adrenalin und Nikotinvergiftung (experimenteller Arteriosclerose) der Kaninchen. Frankfurt Z. Pathol. **36:** 614.

31. Kosdoba, S. A. 1930. Zur Frage nach den pathologisch anatomischen Bergründingen des Zusammenhanges einiger chirurgischer Erkrankungen des Blutgefässsystems mit Nikotismus. Mitt. Grenzgeb. Med. Chir. **41:** 687.

32. Hueper, W. C. 1943. Experimental studies in cardiovascular pathology. VII. Nicotine poisoning in rats and dogs. A.M.A. Arch. Pathol. **35:** 846.

33. Thienes, C. H. 1929. Experiments with nicotine in growing white mice. Am. J. Hyg. **9:** 500.

34. Behrend, A. & C. H. Thienes. 1932. Chronic nicotinism in young rats and rabbits. Effect on growth and estrus. J. Pharmacol. Exptl. Therap. **46:** 113.

35. Thienes, C. H., C. F. Lombard, F. J. Fielding, A. J. Lesser & M. J. Ellenhorn. 1946. Alterations in reproductive functions of white rats associated with daily exposure to nicotine. *Ibid.* **87:** 1.

36. Behrend, A. & C. H. Thienes. 1933. The development of tolerance to nicotine by rats. *Ibid.* **48:** 317.

37. Fields, H. E. Report to the Pacific Coast Division of Am. Assoc. Advancement Sci. Unpublished.

38. Smith, C. S., S. Rosenfeld, Jr. & L. J. Sacks. 1935. Effect of nicotinism in the albino rat. J. Pharmacol. Exptl. Therap. **55:** 274.
39. Thienes, C. H., C. F. Lombard & A. J. Lesser. 1941. Effects of chronic nicotine poisoning on viability of the young and on the adrenal gland. *Ibid.* **72:** 40.
40. Geller, L. M. 1959. Failure of nicotine to affect development of offspring when administered to pregnant rats. Science. **129:** 212.
41. Simpson, W. 1957. A preliminary report on cigarette smoking and the incidence of prematurity. Am. J. Obstet. & Gynecol. **73:** 808.
42. Lowe, C. R. 1959. Effect of mothers' smoking habits on birth weight of their children. Brit. Med. J. **2:** 673.
43. Chiasson, M. J. 1929. Effects of tobacco smoking. J. Am. Med. Assoc. **23:** 1909.
44. Foreign Letters. 1940. Miscarriages among tobacco workers. J. Am. Med. Assoc. **114:** 340.
45. Phillips, L. G. 1943. Cigarette smoking as a factor in sterility. Hawaii Med. J. **2:** 249.
46. Nishimura, H. & K. Nakai. 1958. Developmental anomalies in offspring of pregnant mice treated with nicotine. Science. **127:** 877.
47. Eränko, O., V. Hopsu & L. Räisänen. 1959. Effects of prolonged administration of nicotine on the medullary volume and the distribution of noradrenaline in the adrenals of the rat, the mouse and the guinea pig. Endocrinology. **65:** 293.
48. Essenberg, J. M. 1952. Chronic poisoning of endocrine glands of female albino mice by cigarette smoke. Western J. Surg. Obstet. Gynecol. **60:** 635.
49. Thienes, C. H. & E. M. Butt. 1938. Circulatory effects of nicotine and tobacco. Am. J. Med. Sci. **195:** 522.
50. Doll, R. & A. B. Hill. 1954. The mortality of doctors in relation to their smoking habits. Brit. Med. J. **1:** 1451.
51. Hammond, E. C. & D. Horn. 1958. Smoking and death rates. Report on forty-four months of follow-up of 187,783 men. J. Am. Med. Assoc. **166:** 1294.
52. Buechley, R. W., R. M. Drake & L. Breslow. 1958. Relationship of amount of cigarette smoking to coronary heart disease mortality rates in men. J. Am. Med. Assoc. **169:** 1085.
53. Haag, H. B. & H. R. Hammer. 1957. Smoking habits and mortality among workers in cigarette factories. Ind. Med. and Surg. **26:** 559.

NICOTINE AND SMOKING ON THE DOG BALLISTOCARDIOGRAM*

Aldo N. Corbascio† and James W. West
Departments of Therapeutic Research and of Pharmacology, University of Pennsylvania Medical School, Philadelphia, Pa.

Cigarette smoking on the ballistocardiograph (BCG) was introduced by Henderson[1] in 1953 as a provocative test intended to further the diagnostic potentialities of the BCG in the diagnosis of latent coronary artery disease. Davis *et al.*[2] have given this test extensive clinical trials and have obtained considerable evidence in favor of this procedure, which appears to be more useful than exercise. Such clinical application, however, has not been preceded by a study of the ballistic responses of animals to nicotine.

A series of experiments with dogs was therefore undertaken in order to analyze the pharmacodynamic action of nicotine in terms of changes induced in the generation of ballistic forces within the heart and the cardiovascular system as a whole.

Localized responses following selective intracoronary administration of the drug were compared to the effects obtained after systemic administration (intravenous injections or cigarette smoke inhalation). Parallel studies were also performed with dogs in which an acute myocardial infarction had been induced experimentally.

Methods

The present study was conducted on an ultra-low frequency ballistocardiograph (UF-BCG) built especially for acute experiments with dogs. The instrument consisted of a rectangular frame (180 × 50 cm.) of hollow aluminum tubing (1.5-in. diam.) covered by resilient, lightweight canvas. This platform was suspended from the ceiling by means of 4 light steel cables about 3 m. in length. The natural frequency of the BCG was 0.3 cycles per second. Acceleration was transduced directly by means of a variable capacitance accelerometer‡ weighing only 56 gm. The weight of the unloaded platform was about 3.25 kg. (approximately one tenth of the average weight of the dogs used in the experiment). The signal from the accelerometer was fed directly into a DC amplifier and recorded on a 4-channel Sanborn apparatus, which allowed for concurrent monitoring of ECG, systemic blood pressure, right or left atrial pressure, or respiration. Calibration of the instrument was obtained by the ratio: $D/(L \times d)$ = sensitivity (expressed in term of gravities/cm.), in which D is displacement of the bed (cm.), L is length of suspension (cm.), and d is displacement of the pen on the paper.

The dogs used throughout the experiments were especially selected large mongrels or thoroughbred boxers, 30 to 35 kg. in weight, in optimal nutritional condition and with good muscle tone. They were lightly anesthetized with

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† Present address: Radiological Laboratory, University of California Medical Center, San Francisco, Calif.

‡ Constructed by Walter J. Gamble, 854 Gates Pavilion, University Hospital, Philadelphia, Pa.

morphine (3 mg./kg. I.M.) and a mixture of Dial-urethane and Nembutal (0.25 ml./kg.). One femoral artery and the contralateral femoral vein were cannulated for blood pressure recording and injection, respectively. The trachea was exposed and cannulated or intubated with a dog tracheal tube. A rigid West coronary catheter was introduced fluoroscopically through the common carotid into the anterior descending branch of the left coronary artery for direct intracoronary injection of drugs, according to a previously developed technique by West[3] that allows selective catheterization of all three main coronary branches (anterior descending, left circumflex, and right coronary). This technique offers some unique advantages, as it allows introducing drugs into the arterial supply of the left ventricle alone, leaving all the other sections of the vasculature unaffected. It was thus possible to single out the left ventricular components of the cardiac action of nicotine on the BCG with a selectivity previously impossible. It also circumvented the necessity of using open-chest preparations, which are undesirable because the operative procedure is poorly tolerated and often induces irreversible deterioration of the ballistocardiogram.

After coronary catheterization, the animal was transferred to the UF-BCG, placed on its right side, and its limbs were secured to the bed by means of conventional leather straps while wide canvas belts were tightened across the chest and abdomen to insure maximum coupling to the bed. Spontaneous respiration was stopped by means of decamethonium bromide (0.2 mg./kg. I.V.) while an adequate respiratory exchange was maintained by means of an Ideal Starling pump connected to a tracheal cannula or tube. The connections were such that no appreciable drag was exerted on the bed. Neuromuscular blockade eliminated all tremors and muscular twitchings, thus insuring an almost artifact-free record. Recordings were obtained during temporary apnea as well as during assisted respiration. No artifact or base-line weave was seen in either case.

Results

After an initial control tracing had been secured, a small dose of nicotine bitartrate (0.5 μg./kg.) was injected into the anterior descending branch of the left coronary artery. The injection of the drug at a site which selectively distributes it to the anterior part of the left ventricular muscle was followed within 3 or 4 sec. by a striking increase in amplitude of the ventricular components of the systolic complex (I–J) on the ballistocardiogram (an increase of more than 50 per cent of the control value) and a marked change in slope of the H–I and I–J waves with sharpening of their respective peaks (FIGURE 1). This effect lasted 10 or 15 sec., after which the tracing gradually reverted to its control amplitude. Systemic blood pressure and heart rate remained almost unaffected. A striking change occurred instead in the chest and peripheral leads of the ECG, in which the T wave was greatly increased in height. Such ECG changes are elicited particularly by intracoronary injection of sympathomimetic amines which have a cardiac stimulating action. No convincing explanation is yet available for this modification of the repolarization wave of the ECG. The slight pressure rise (∼5 mm. Hg) occasionally observed under these circumstances was probably due to a transitory increase in cardiac stroke volume induced by nicotine, which in previous experiments[4] has been found capable of augmenting the contractility of the ventricular muscle. This definite, positive inotropic response could be elicited at will in the course of the same experiment, provided that the injections were spaced 15 min. apart. The intracoronary injection (0.25 to 0.5 μg./kg.) of DMPP (I-I-dimethyl-4-phenylpiperazinium iodide), a synthetic congener of nicotine[5] that has the same ganglionic effects, gave identical responses with an appreciably smaller dose (FIGURE 2).

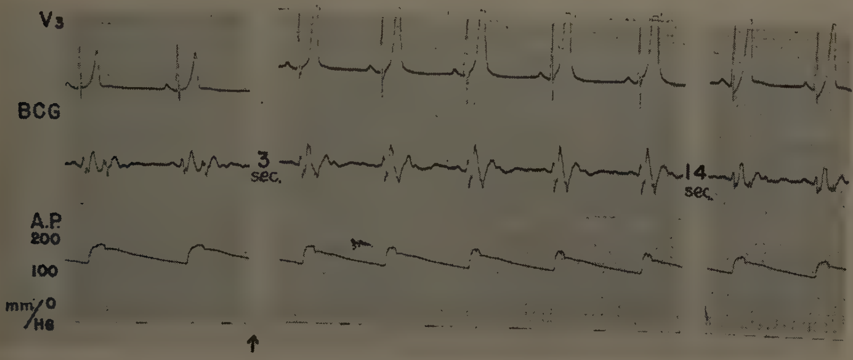

FIGURE 1. Note the profound modification of the systolic complexes of the ballistocardiogram (I–J increased 98 per cent above control) after an intracoronary injection of nicotine (0.35 μg./kg.) into the left anterior descending branch. The arrow indicates the time of injection. T wave in the chest leads of the ECG is also affected while P and H are unmodified. Heart rate and systemic blood pressure are likewise unchanged, although the ascending limb of the aortic pressure wave has a greater slope than the control. Other studies have indicated that this is due to a change in left ventricular ejection and an increase in myocardial contractility.

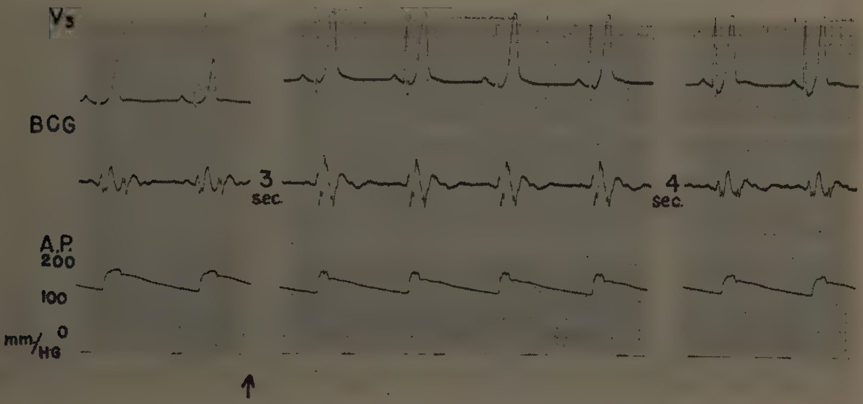

FIGURE 2. The ballistocardiographic and electrocardiographic response to intracoronary administration of DMPP (0.17 μg./kg.) into the left anterior descending branch is identical to the response to nicotine, except that a smaller dose produces a longer-lasting effect.

The effects of intracoronary administration of nicotine or DMPP were also studied in dogs after a large number of small emboli had been introduced into the anterior descending branch of the left coronary artery, to induce a lesion similar to an acute anterior myocardial infarction. This was accomplished by repeated injections through the coronary catheter of 0.1 to 0.2 ml. of a 1 per cent suspension of lycopodium spores (average diameter 60 μ) in saline solution. This procedure was followed by a progressive distortion of the systolic complexes of the ballistocardiogram, while the S-T segment of the ECG (V_3 lead) showed the typical changes of acute anterior myocardial infarction, and systemic blood pressure and pulse pressure gradually declined to shock levels.

Intracoronary injections of drugs were made several hours after the circulatory shock consequent to acute infarction had been induced, at a time when the ballistocardiogram was grossly abnormal in form and small in amplitude. In spite of this myocardial damage, the response to nicotine and DMPP was practically identical to that seen in the non-infarcted animals. After administration of the drugs, the systolic complexes of the ballistocardiogram appeared to be substantially improved; the I–J wave, which could not be clearly detected in the post-embolization control record, reappeared at full amplitude, and the entire ballistic picture was reverted to normal temporarily. No qualitative differences could be noted between the two drugs; DMPP, however, appeared to be almost twice as potent in restoring contractility in the infarcted heart and the duration of its effect outlasted that of the naturally occurring alkaloid.

The effects elicited by intracoronary nicotine and DMPP were compared to the responses obtained by the intravenous injection of the two drugs in the course of the same experiment. Intravenous administration of nicotine in doses of about 5 μg./kg. produced a radically different picture (FIGURES 3 and 4), which is characteristic of the systemic action of the two drugs. After a period of 10 to 15 sec. following injection, systemic blood pressure increased sharply (as much as 50 mm. Hg above the control value), whereas the amplitude of the systolic complexes of the ballistocardiogram (particularly the I–J wave) was reduced, and the entire record reflected a decrease in the generation of ballistic forces. This first phase of the response lasted a variable period of time (from 20 sec. to 3 min.), after which the systolic pressure and the ballistic record reverted to their original form and amplitude. Later on, however, the situation appeared to be completely reversed, with a definite improvement in amplitude of the systolic complexes of the ballistocardiogram in spite of the persisting increase in systemic arterial pressure, pulse pressure, and heart rate. In this second phase, the over-all amplitude of the systolic complexes of the ballistocardiogram (H–I–J–K) was increased well above 50 per cent of the control. This picture of stimulation was of longer duration (up to 60 sec.) than the initial phase of depression.

Another series of experiments was undertaken to study the ballistic effects of nicotine inhaled with the smoke from a common-brand cigarette (no filter). This was accomplished by inserting a lighted cigarette into the air intake of the respiratory pump used to maintain the dog's respiration. The rate of the pump was about 30 cycles/min., and the stroke volume was usually kept around 250 ml. of air. The cigarette smoke was administered in a series of 10 to 15 inhalations, after which the cigarette was disconnected. Ballistocardiogram, ECG, femoral arterial pressure, and right atrial pressure were monitored as

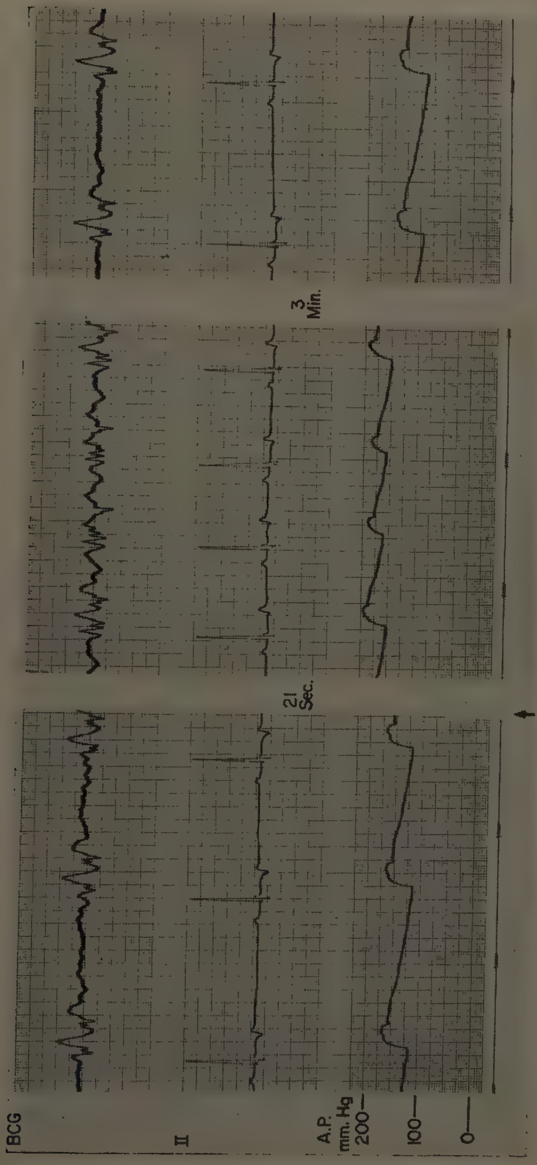

FIGURE 3. Note the alteration induced in the ballistocardiogram by the intravenous injection of nicotine (5 μg./kg.). The ballistocardiographic record is at its worst at the height of the blood pressure rise.

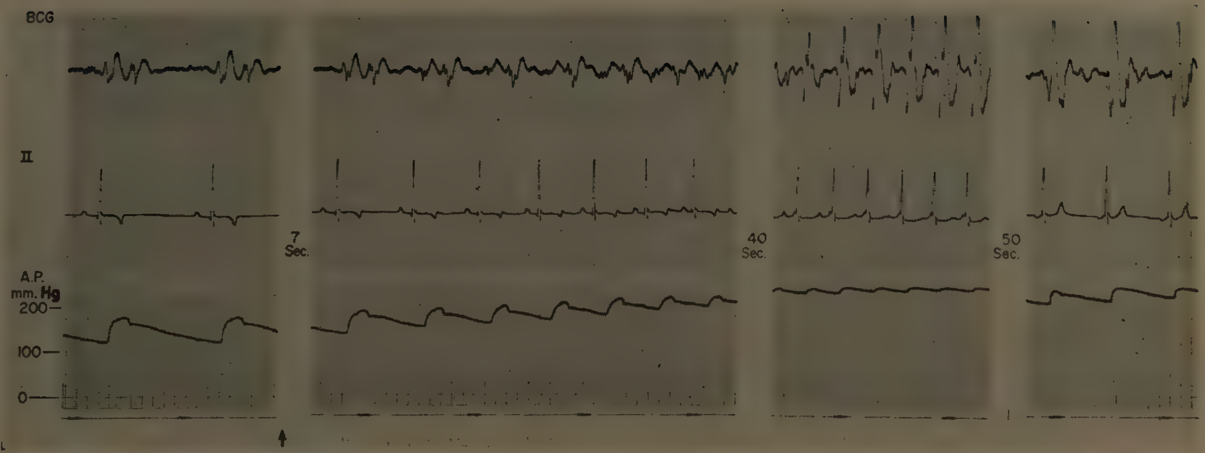

FIGURE 4. The action of DMPP (5 μg./kg. I.V.) shows initially an even more deleterious effect than nicotine on the ballistocardiogram. As soon as stimulation of cardiac ganglia takes place, however, the ballistocardiographic tracing improves in spite of the high pressure in the aorta. Fifty seconds later baroceptor activation induces cardiac slowing. The tracing is further improved owing to better filling of the heart and increased stroke volume.

usual during the inhalation and the ensuing three minutes. As shown in FIGURE 5, the effects obtained were practically identical to those seen after the injection of the pure alkaloid or its synthetic congener. The only noticeable difference was the slower and more gradual onset of the response and its longer duration. It was evident that both phases of the nicotine effect (ballistocardiographic deterioration followed by improvement) could be elicited by this means.

Discussion

The direct coronary application of nicotine and DMPP to the anterior descending branch of the left coronary artery produced a consistent picture of stimulation of the left ventricular muscle and a definite improvement in the ventricular components of the ballistocardiogram (I–J wave). This response was qualitatively similar to that obtained with smaller doses of epinephrine or norepinephrine injected at the same site. Liberation of the sympathetic neurohumoral mediator from cardiac ganglia has been shown to account for this positive inotropic effect of nicotine.[6] It is also known that sympathomimetic amines appear in significant quantities in the perfusate of isolated mammalian hearts infused with nicotine.[7] This has been verified by biological assays on the hypodynamic isolated frog heart, the fowl cecum, and on the isolated rabbit intestine preparation, as well as by spectrofluorometric measurements. Previous studies by West and co-workers[4] in open chest dogs have also shown that intracoronary injections of nicotine induce an increase in myocardial contractility measured with the Walton strain gauge applied directly to the

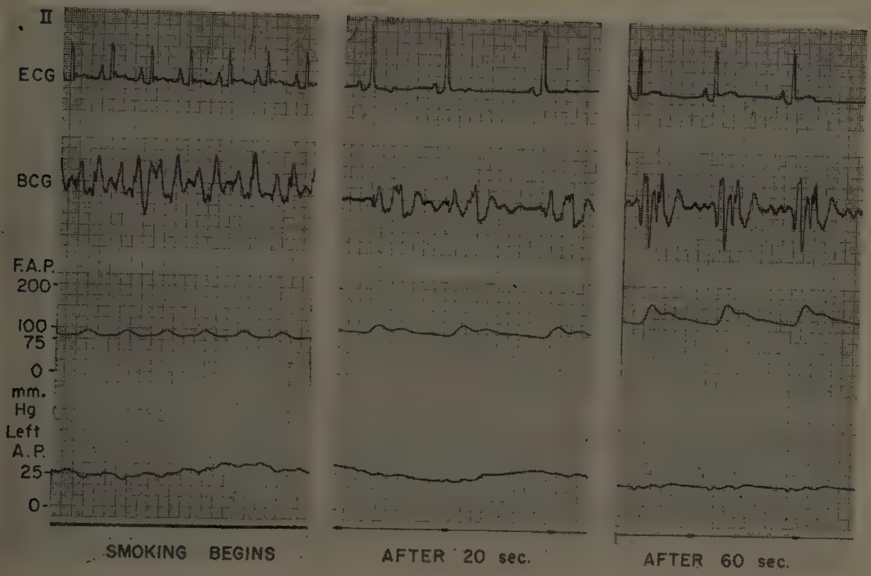

FIGURE 5. The ballistocardiographic tracing was adversely affected by cigarette smoke during the first 20 to 40 sec. of exposure; 60 to 120 sec. later, it was definitely improved.

left ventricle, as well as an increase in oxygen extraction and coronary blood flow.

The systemic response to intravenous injection of nicotine and DMPP appeared therefore to be diphasic. The initial phase was dominated by a systemic pressor response, with deterioration of the ballistic tracing (in spite of the occasional slowing of the heart which normally tends to increase the amplitude of the ballistic record as stroke volume adjusts its output to the slower rate). This initial effect was considered to be peripheral in nature and due to stimulation of the sympathetic autonomic ganglia. The consequent increase of peripheral resistance and modification of left ventricular ejection with decreased ability of the heart to accelerate its content could account for the adverse effects on the ballistocardiogram. The change in shape of the ejection curve that ensues is reflected by the decrease in slope of the I–J components of the ballistocardiogram.

After 15 to 20 sec. the record suddenly improved in spite of the elevated systemic blood pressure. This secondary effect was considered to be the central or cardiac component of nicotine action: the drug had acted on the cardiac sympathetic ganglia and elicited therefore a picture of central stimulation of the heart, with positive chronotropic and inotropic effects. Stroke volume was increased, ejection was more rapid and complete, and the amplitude of the ballistic record exceeded the amplitude of the control, while right atrial pressure was unchanged. This eliminated the possibility that such change in contractility might be due to an increased venous return and consequent increase in filling pressure of the heart. The improvement in the ballistocardiographic tracing was even more remarkable in face of the high pressure already existing in the arterial system. This would indicate that cardiac contractility was enormously increased, as the high pressure in the aorta would tend, by opposing the ejection of blood by the heart, to modify the shape of the ejection curve, thus diminishing the amplitude and decreasing the slope of the I–J wave. It is reasonable to assume that, besides its direct effect on the heart, sympathoadrenal discharge played a considerable role in the production of the stimulatory phase. This, however, has not been fully investigated.

It is interesting to note that such a biphasic picture of peripheral vascular stimulation with ballistic deterioration, immediately followed by heart stimulation and improvement, could be faithfully reproduced by inhalation of cigarette smoke. This finding is of substantial value for the interpretation of the smoking test on the BCG as currently practiced in the clinic. This would indicate that the initial contention according to which nicotine can be used as a circulatory stressor agent applies only to the initial effect of the drug (hypertension without cardiac stimulation). In cases in which the timing of the test has not been accurate, the later phase of cardiac stimulation could erroneously lead to the conclusion that the patient's heart is unimpaired and that the test is therefore negative. The fact that a ballistic improvement can be elicited with nicotine in animals that have been subjected to acute myocardial infarction should induce further caution in the interpretation of clinical results.

These findings suggest a revision of the current technique for performing the smoking test on the BCG, with constant monitoring of systemic blood pressure as a prerequisite.

Clinicians are accustomed to consider nicotine as invariably detrimental to the heart and vessels. The smoking test on the BCG relies on nicotine as a circulatory stressor for detecting latent myocardial disabilities. Our results seem to contradict this concept: it has been shown that nicotine injected directly into the coronary vessels acts as a powerful stimulant, while the systemic administration of the drug is only initially attended by cardiac depression, soon to be followed by myocardial stimulation. Which of these actions prevails in humans under normal or pathological conditions is not clear. One would expect both effects to be elicited under certain conditions in patients undergoing the test, and would anticipate that a more continuous and prolonged period of observation after cigarette smoking might confirm the occurrence of a biphasic response to cigarette smoking in man.

Summary

The ballistic effects of nicotine and its synthetic congener DMPP have been studied in lightly anesthetized intact-chest dogs. The effects of systemic intravenous and of intra-arterial injections in the anterior descending branch of the left coronary artery were compared. Both drugs applied locally to the heart induced a definite positive inotropic response in the ventricular musculature. The response attending systemic administration was essentially biphasic, with an initial pressor rise inducing adverse ballistic effects, followed by signs of cardiac stimulation (positive chronotropic and inotropic effects) with improvement of the ballistocardiogram.

This was also verified with dogs subject to acute anterior myocardial infarction. Inhalation of cigarette smoke faithfully reproduced the effects of systemically injected nicotine.

The data thus obtained suggest a revision of the clinical criteria for interpreting the smoking test on the BCG and a modification of its performance that may improve its diagnostic validity.

Acknowledgments

We acknowledge the generous support and the enlightened guidance of Isaac Starr, University of Pennsylvania Medical School, and the collaboration of his staff: A. Noordergraaf for his suggestions concerning the physical characteristics of the UF-BCG, Walter Gamble for providing a highly efficient accelerometer, and George Pierce for details concerning the assembly and operation of the apparatus.

References

1. Henderson, C. B. 1953. Ballistocardiograms after cigarette smoking in health and in coronary artery disease. Brit. Heart J. **15:** 287.
2. Davis, F. W., W. R. Scarborough, R. E. Mason, M. L. Singewald & B. M. Baker. 1956. The ballistocardiographic cigarette test: further observations. Am. Heart J. **51:** 165.
3. West, J. W., T. Kobayashi & S. V. Guzman. 1958. Coronary artery catheterization in the intact dog. Circulation Research. **6:** 383.

4. West, J. W., S. V. Guzman & S. Bellet. 1956. Cardiac effects of intracoronary arterial injections of nicotine. Circulation. **14:** 1017.
5. Chen, G., R. Portman & A. Wickel. 1951. Pharmacology of I-Dimethyl-4-Phenyl-Piperazinium iodide, a ganglion stimulating agent. J. Pharmacol. Exptl. Therap. **103:** 330.
6. Burn, J. H. & M. J. Rand. 1958. Action of nicotine on the heart. Brit. Med. J. **1:** 137.
7. Richardson, J. A. & E. F. Woods. 1959. Release of nor-epinephrine from the isolated heart. Proc. Soc. Exptl. Biol. Med. **100:** 149.

EFFECT OF CIGARETTE SMOKING ON THE INTRAOCULAR BLOOD VOLUME*

Victor G. Fellows and Jerome W. Bettman
Department of Ophthalmology, Presbyterian Hospital and Medical Center of San Francisco, San Francisco, Calif., and Division of Ophthalmology, Stanford University Medical School, Palo Alto, Calif.

We have been investigating factors that influence the blood supply of the choroid and retina for a number of years.[1] During the course of these investigations we found that smoking, under certain conditions, and nicotine can have a marked effect on the intraocular blood volume in experimental animals. Some of this work has been reported previously,[2] but it has subsequently been amplified, as will be shown in this paper.

Although a number of different methods have been used to study changes in intraocular blood volume, none has been satisfactory. These may be summarized as follows:

1) Techniques dependent on direct observation of the vessels of the retina. These include direct observation with an ophthalmoscope with or without a grid,[3] photography with a fundus camera,[4,5] and, more recently, television.[6] Although we have had no experience with television, certainly the other methods do not offer sufficient resolving power to permit changes in caliber of the degree anticipated. Furthermore, only the retinal vessels can be observed, and these represent but 1/37 of the total blood volume of the chorioretinal layer.

We are well aware that certain investigators in the past have felt that vasoconstriction after smoking could be detected ophthalmoscopically. Careful study of fundus photographs with the finest grain paper obtainable, examined critically under a dissecting microscope, failed to reveal any such change.

2) Technique of direct observation of the blood vessels of the choroid: the scleral window technique of Leopold.[7] This method involves the insertion of a plastic button under the sclera, removal of the overlying sclera, and observation of the underlying choroidal blood vessels. In our hands it has been completely unsatisfactory (12 animals); it involves considerable trauma, can be used only in an albinotic animal, and permits only examination of the larger choroidal vessels. No response could be elicited from most agents that resulted in a marked effect by our radioisotope techniques (see below).

3) Techniques dependent upon secondary physiological effects resulting from changes in vascularity. These include electroretinography,[8,9,10,11] tests of flicker fusion frequency,[12] angioscotometry and changes in intraocular tension. All of these methods except the last can be influenced by changes in the retinal synaptic functions,[13] as well as by vascular changes and, therefore, are not reliable. Changes in intraocular tension are induced by so many factors that these are difficult to evaluate. During a study of the usefulness of P^{32} in the diagnosis of intraocular melanomas we came to recognize that this isotope

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might provide a useful tool as an indicator of changes in the volume of blood in the choroid and retina.

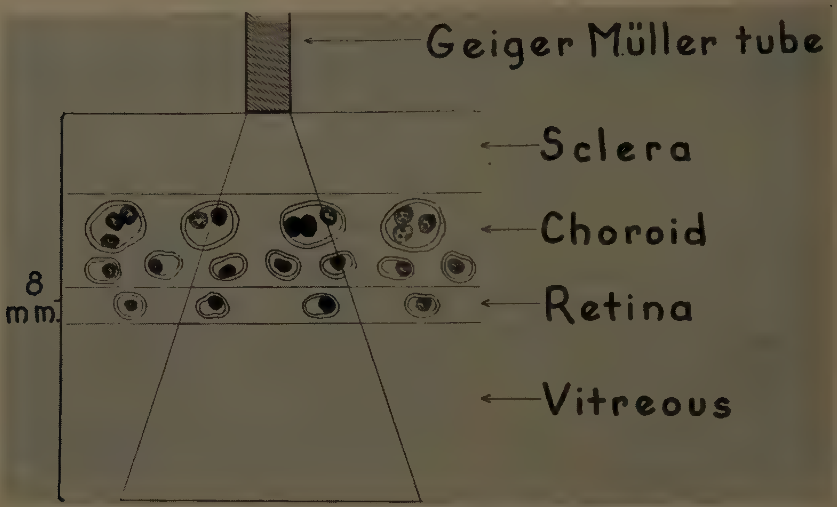

FIGURE 1. Measurement of radioactivity from choroid and retina.

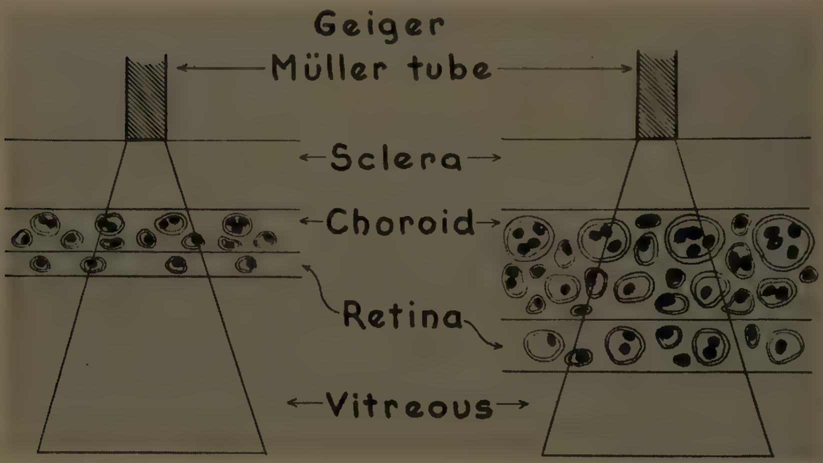

FIGURE 2. Increase in blood volume, resulting in more radioactivity under Geiger-Müller tube.

We adapted to ophthalmic use a method reported by Berson and Yallow.[14] Briefly, the technique is as follows:[15]

Ten cc. of blood are drawn from the experimental animal, heparinized, and mixed with 2.0 mc. of P^{32}. The mixture is incubated in a rubber-stoppered centrifuge tube at 37° C. for two hours and, during this time, it is gently agitated. The plasma and buffy coat are removed, and the labeled RBC are then centrifuged and washed three times with isotonic saline. The third washing is sufficient to reduce the radioactivity outside of the RBC's to approximately 1 per cent of the total. The radioactive resuspended red blood cells are then reinjected into the experimental animal.

The radioactive phosphorus is almost entirely confined to the red cells, which are all within the vascular tree; hence the radioactivity must depend upon the volume of blood in the blood vessels within range of the detecting Geiger-Müller tube.

This may be clarified by reference to FIGURE 1. If the Geiger-Müller tube is placed on the bare sclera at a point posterior to the ora serrate, the tissues within 8 mm. of it are the sclera, the choroid, the retina and the vitreous. As the β particles of P^{32} only penetrate a maximum of 8 mm. through tissue, and as P^{32} emits only β particles, we need only concern ourselves with the tissues that lie within 8 mm. The vitreous contains no blood vessels; the sclera, very few. Almost all the blood is contained in the choroid and retina, especially in the former. As illustrated in FIGURE 2, if the volume of blood in the vessels of the choroid and retina is increased, more radioactivity will be detected by the Geiger tube.

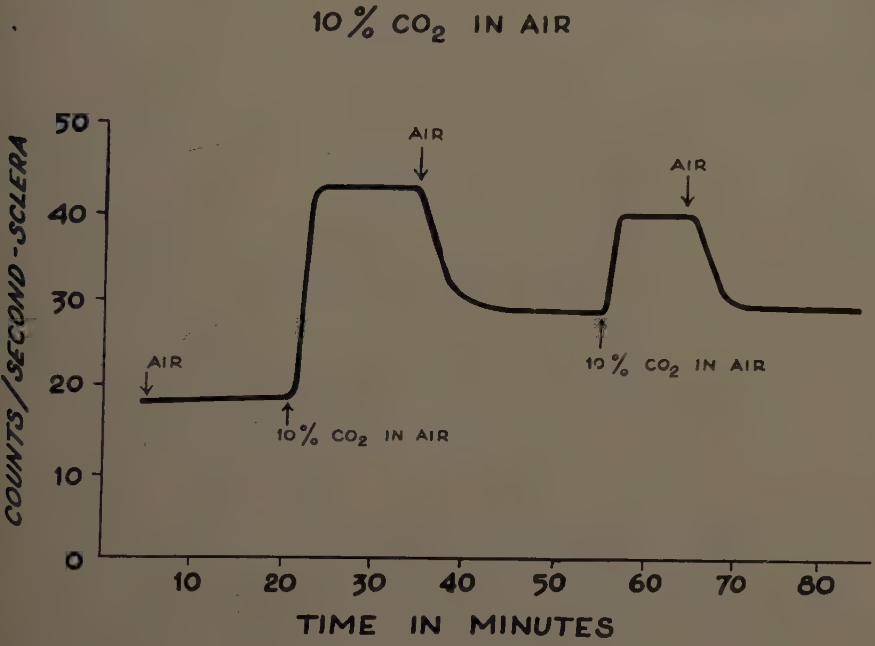

FIGURE 3.

It was necessary to regulate the respirations by tracheotomizing the animal and connecting a cannula in the trachea with a respiratory pump so that the exchange of air could be regulated. If the CO_2 were allowed to accumulate, as in an animal that held its breath, the blood volume was increased by the vasodilator action of CO_2. This effect is illustrated by administering 10 per cent CO_2 in air as in FIGURE 3 (10 animals).

During these investigations a lighted cigarette was placed in the intake tube of the breathing machine. If the cigarette were smoked with occasional puffs, as in leisurely smoking (smoked in 7 to 10 min.), no effect on the intraocular blood volume as determined by radiation was noted (FIGURE 4) (4 animals).

However if the cigarette was smoked extremely rapidly, a marked increase in blood volume was obtained, as illustrated in FIGURE 5. This effect was of short duration and ceased almost as soon as the cigarette burned itself out.

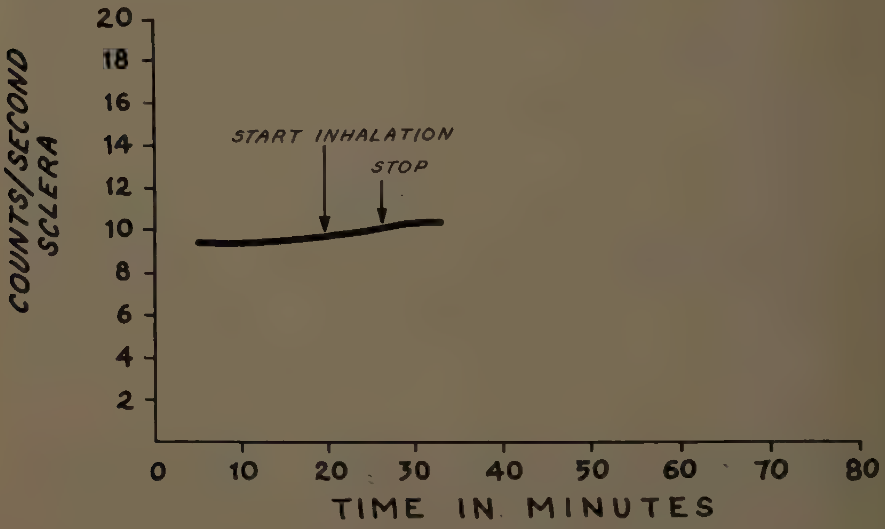

FIGURE 4. Effect of slow smoking of a cigarette.

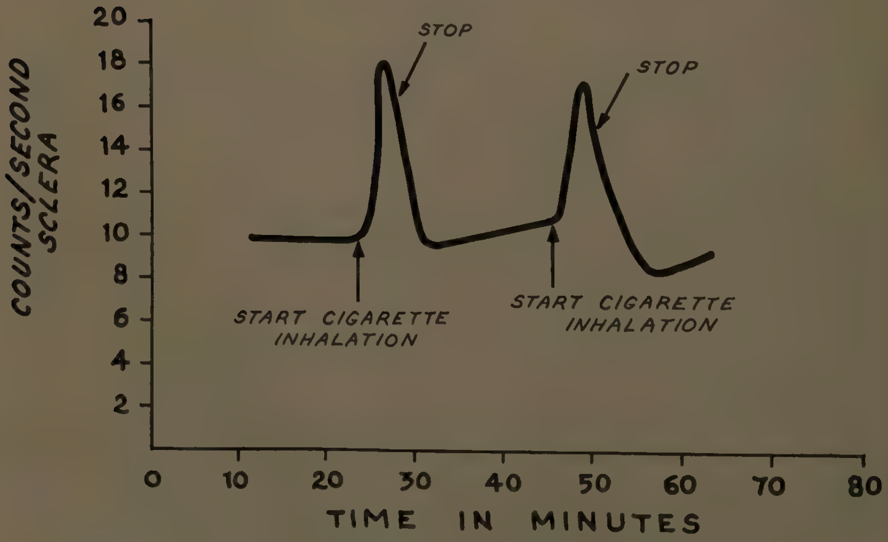

FIGURE 5. Effect of rapid smoking of a cigarette.

The effect was repeatable a short time later, as illustrated in the figure (8 animals).

We obtained the same effect from the injection of nicotine solution intravenously, as shown in FIGURE 6. This observation, that the vascular effect of smoking was probably due to the nicotine, corresponds to statements of others. The other constituents of smoke, such as pyridine base, carbon monoxide, and ash of the cigarette paper, produce no such effect.[16]

If a cigarette with part of the nicotine extracted (Sano brand was used) and more nicotine filtered out by use of a Denicotea filter was smoked at a moderate rate, no change occurred, but if it was smoked rapidly (in 2 to 4 min.) some increase in blood volume still was obtained (4 animals).

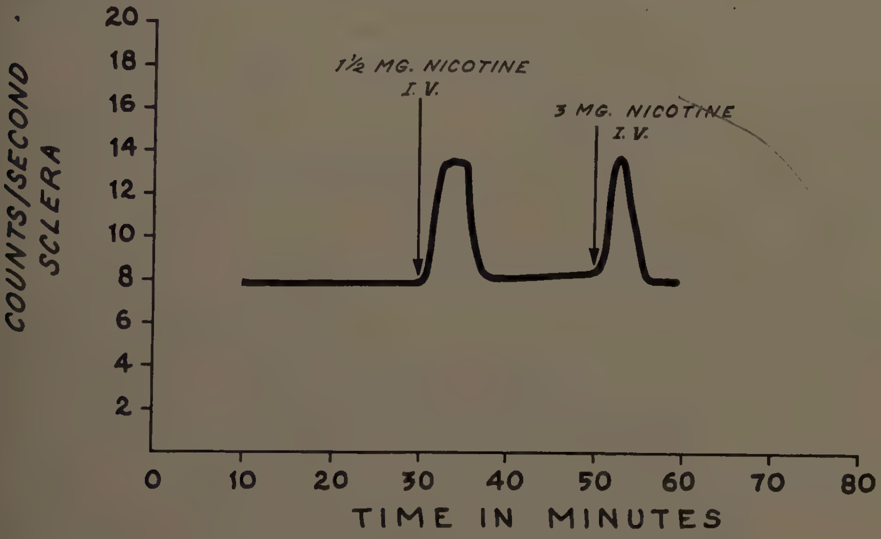

FIGURE 6. Effect of injection of nicotine solution.

If an ordinary commercial cigarette was impregnated with an additional 20 mg. of nicotine, and then smoked at the usual casual rate a variable increase in blood volume did occur in 13 out of 15 animals.

The effect of pipe smoking and cigar smoking was less marked, although some effect was obtained. This lesser effect with pipe and cigar smoking may well be because the pipe simply didn't smoke as fast.

It is probable that the increase in blood volume in the eye is secondary to peripheral vasoconstriction and, in this sense, it is a passive dilation. We have previously demonstrated that the effect of a number of drugs is secondary to, and hence the opposite of, the effect on the peripheral vessels.[1] As examples, acetylcholine and methacholine (Mecholyl), although peripheral vasodilators, decreased the intraocular blood volume both when given systemically or by the retrobulbar route. Epinephrine produced a definite increase in blood volume either when given systemically or by the retrobulbar route. The change in intraocular blood volume seemed secondary to the peripheral changes as it occurred with other signs of systemic effect, such as salivation with methacholine, and rise in blood pressure and faster pulse with epinephrine.

To determine whether the effect of smoking on the intraocular blood volume was secondary to peripheral vasoconstriction we administered 0.4 mg. of a peripheral vasodilator, Nylidren HCl (Arlidin), and followed this in a short interval by the same rapid smoking of a cigarette that had produced a marked increase of blood volume of short duration. When this same type of smoking was performed after Nylidren some increase in blood volume in the eye still occurred, but the effect was less marked and definitely more prolonged (8 animals). The increase was diminished by approximately one half or less, and the effect was prolonged to at least twice the original duration.

We attempted to correlate skin temperature measurements with a needle thermocouple under the skin with these changes. The results up to this time are too variable to be significant. Further work remains to be done on this difficult problem of the peripheral blood volume and correlating it with changes in the intraocular blood volume.

At this time our work indicates that very rapid smoking or injection of nicotine increases the volume of blood in the choroid and retina in the cat. This effect is probably secondary to peripheral vasoconstriction and can be diminished by preliminary administration of a vasodilator.

The vasodilation is not obtained on casual smoking.

It is too early to reach conclusions concerning the clinical application of this phenomenon in man. One cannot help but wonder, however, if many vasodilators that have been administered in enormous quantities in an attempt to increase the intraocular blood supply do not in fact diminish it, as a sequel to peripheral vasodilation. It is possible that the drugs of choice to use systemically would be peripheral vasoconstrictors if one wished to increase intraocular blood volume.

References

1*a*. Bettman, J. & V. Fellows. 1956. Factors influencing the blood volume of the choroid and retina. Trans. Am. Acad. Ophthalmol. Otolaryngol. **60**(6): 791–805.
1*b*. *Ibid.* 1958. Am. J. Ophthalmol. **46**(1 Part 1): 1–10.
2. Bettman, J., V. Fellows & P. Chao. 1957. The effect of cigarette smoking on the intraocular circulation. Trans. Pac. Coast Oto-Ophthalmol. Soc. **38:** 39–57.
3. Lambert, R. 1932. A simplified retinal measuring grid. A.M.A. Arch. Ophthalmol. **7:** 440–443.
4. Lambert, R. 1934. A method for the study of the retinal circulation. A.M.A. Arch. Ophthalmol. **12:** 868–873.
5. Puntenny, I. 1939. Effect of certain stimuli on the calibre of retinal blood vessels. A.M.A. Arch. Ophthalmol. **21:** 581–597.
6. Potts, A., S. West & J. Shearer. 1959. Application of television ophthalmoscope. A.M.A. Arch. Ophthalmol. **62:** 485–499.
7. Leopold, I. 1951. Autonomic drugs and their influence on choroidal vessel calibre. Trans. Am. Ophthalmol. Soc. **49:** 625–672.
8. Henkes, H. 1951. The use of electroretinography in measuring the effect of vasodilation. Angiology. **2:** 125–131.
9. Heckman, J., J. Shieve & W. Wilson. 1953. The relation between retinal and cerebral vascular reactivity in hormonal and arteriosclerotic subjects. Circulation. **7:** 84–87.
10. Lambert, J. & M. Lincoln. 1954. Effect of vasodilation drugs and stellate ganglion block upon the electroretinogram. A.M.A. Arch. Ophthalmol. **52:** 917–922.
11. Karpe, G. 1945. The basis of clinical electroretinography. Acta Ophthalmol. **23**(Suppl. XXIV).

12. Scher, J. & W. Spankus. 1955. Flicker fusion frequency and hypertension. Am. J. Ophthalmol. **39:** 705–715.

13. Goodman, L. & A. Gilman. 1955. The Pharmacological Basis of Therapeutics. 2nd ed. : 626. Macmillan. New York, N. Y.

14. Berson & R. Yalow. 1952. The use of K^{42} or P^{32} labeled erythrocytes and I^{131} tagged human serum albumin in simultaneous blood volume determinations. J. Clin. Invest. **31:** 581–591.

15. Bettman, J. & V. Fellows. 1946. A technique for the determination of blood volume changes. Am. J. Ophthalmol. **42**(4 Part II): 161–166.

16. Roth, G. 1951. Tobacco and the Vascular System. Thomas. Springfield, Ill. Publ. No. 100. Am. Lecture Ser.

CIRCULATORY RESPONSES TO SMOKING IN HEALTHY YOUNG MEN

Caroline Bedell Thomas and Edmund A. Murphy
The Johns Hopkins University School of Medicine, Baltimore, Md.

Individual variation in the effects of smoking upon the circulation has long been recognized, particularly by smokers themselves. The possible significance of such diversity, however, has not been systematically explored. The circulatory responses of normal persons have been compared with the findings in patients with coronary heart disease, but little attention has been paid to the possibility that the varying reactions to smoking found among healthy young adults are expressions of basic differences in the physiology of the individual which are important in terms of future health, on the one hand, or future cardiovascular disease, on the other. Accordingly, we are observing the circulatory effects of smoking in Johns Hopkins medical students as part of a long-term investigation concerning the precursors of hypertension and/or coronary heart disease. It is our purpose to determine whether the circulatory response to smoking is of predictive value in regard to the development of hypertension or coronary disease.

All smoking tests were performed on a Starr-type ballistocardiograph, so that stroke volume and cardiac output could be studied in conjunction with blood pressure and heart rate. The frequency of deterioration in ballistocardiographic form among patients with coronary disease, compared with control subjects, has already been discussed in this monograph. Gross changes in the records of many coronary patients and of some normal subjects over 30 years of age make it difficult or impossible to measure and compare changes in stroke volume and cardiac output that occur in these groups after smoking. In healthy young adults, however, good ballistic form is found with very few exceptions, presenting an excellent opportunity for quantitative measurements of stroke volume and cardiac output both before and after smoking through the use of Tanner's formula.

The ballistocardiographic smoking test, as we carry it out, is as follows. After being weighed and measured, the subject lies on the ballistocardiographic table. Successive blood pressure measurements and pulse rates are taken until stable. The control ballistocardiogram is then recorded. The subject is given a standard cigarette to smoke at his own pace, inhaling if possible. When two thirds of the cigarette has been smoked, another blood pressure and ballistocardiogram are taken. A second cigarette is smoked in similar fashion and the various measurements repeated.

We are primarily interested in the *change* that follows smoking a cigarette in six variables: systolic pressure, diastolic pressure, pulse pressure, heart rate, stroke volume, and cardiac output. The mean pattern of circulatory response to smoking a single cigarette is shown in FIGURE 1. The bars indicate the mean percentage changes for each of the six variables among the first 113 subjects tested.[1] Although none of the mean changes is very large, all are significantly different from zero. On the average, systolic pressure, diastolic pressure, heart rate, and cardiac output increase after smoking, while pulse pressure narrows and stroke volume diminishes. These effects are seen after the first cigarette; negligible additional changes follow smoking a second cigarette. Accordingly, most of our studies are concerned with the effect of the first cigarette. The direction and degree of change after smoking vary greatly from subject to subject, so that the range of values about the mean for each circulatory measurement is very wide.

When individual patterns of circulatory response to smoking are examined, they show striking differences (FIGURE 2). The first subject (56M67) shows well-marked increases in all six variables, five of them amounting to 22 to 41 per cent of the resting values. In contrast, the pattern of the subject directly below (53M08) shows almost no change in any determinant. Another subject (53M42) responds with a sharp increase in pulse pressure, while below is one (54-M78) who reacts with an equally marked decrease in pulse pressure. Whereas the heart rate usually increases as the stroke volume decreases, as in subject 53M47, in subject 54M45 just the reverse occurs. These variations in individual smoking patterns aroused our curiosity and led us to make a number of subsidiary studies in an effort to determine their meaning.

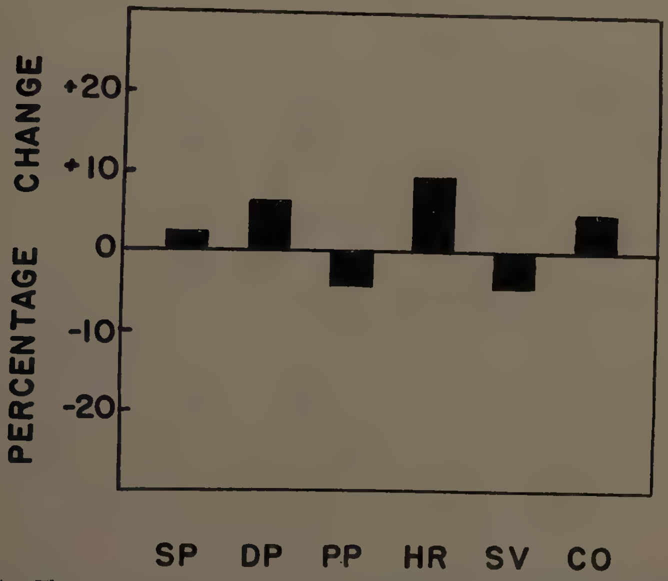

FIGURE 1. The pattern of mean circulatory changes of 113 healthy young adults in response to smoking one cigarette. SP = systolic pressure; DP = diastolic pressure; PP = pulse pressure; HR = heart rate; SV = stroke volume; CO = cardiac output. Reproduced by permission of *Annals of Internal Medicine*.[1]

The reproducibility of the ballistocardiographic smoking test was investigated by observing six male smokers in a series of eight tests each, under uniform test conditions on different days.[2] The circulatory patterns of response for the six subjects are shown in FIGURE 3, where each column represents the mean percentage change in eight tests. It will be seen that marked individual differences in response are not abolished when as many as eight tests per subject are averaged. This is a different group of subjects from those shown in FIGURE 2. Persons of varying degrees of reactivity were purposely included in this study. In general, they maintained different characteristic patterns of reactivity throughout the series of tests. From the average standard deviations we have estimated the reliability of any single test in one subject, and found that the circulatory changes on smoking as measured in the first test are an unbiased estimate of the mean. Although the mean of results in repeated tests would give more information than a single test, nevertheless the first test is as accurate and informative as any other single test. This conclusion fulfills a most important requirement for a test, which is to be useful as a screening procedure.

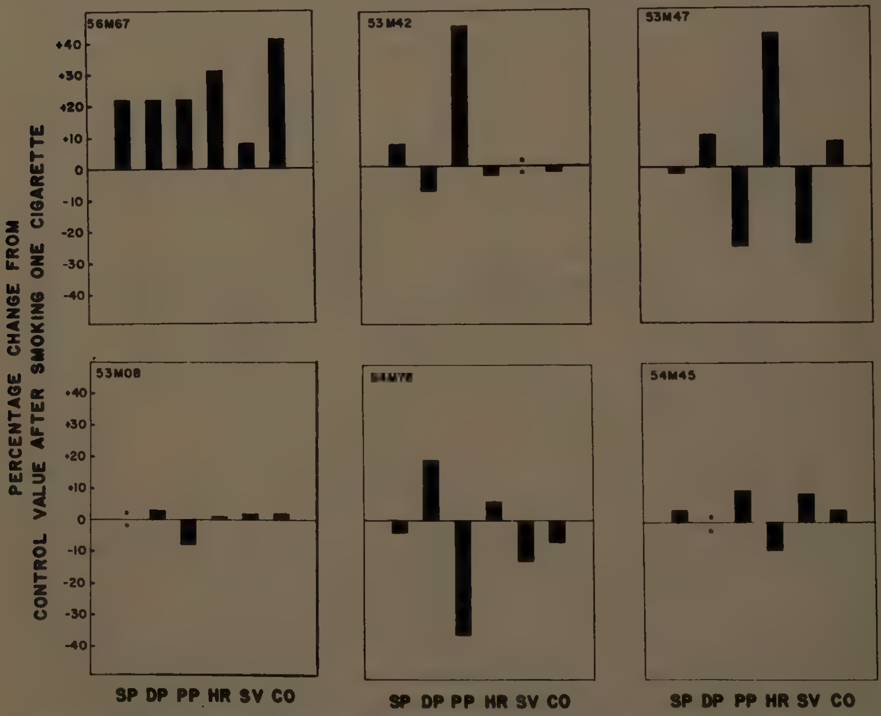

FIGURE 2. Six different individual patterns of circulatory response to smoking. Each pattern represents the percentage changes in six variables shown by a single subject immediately after smoking one cigarette, compared with his own control values. (Key as in FIGURE 1.) Reproduced by permission of *Annals of Internal Medicine*.[1]

The intraindividual variation in the eight smoking tests on each of six subjects is shown in FIGURE 4. The black circles mark the mean value and the lines, one standard deviation above and below it. F represents the ratio of interindividual to intraindividual variance and is an indication of the confidence with which subjects may be classified according to the magnitude of their response. The analysis of variance shows that intraindividual variations of systolic pressure, diastolic pressure, heart rate, and cardiac output were significantly smaller than interindividual variations. This separation is an even more important requisite for a screening test because it makes it possible with fair confidence to assign subjects to one of several broad groups, according to the magnitude and direction of the response. These findings support the view that individual differences in the patterns of circulatory change on smoking have real meaning and are not artifacts due to variance. It is worth noting, however, that the scatter of the results within the individual varies appreciably from subject to subject, so that confidence limits may be applied safely to groups only.

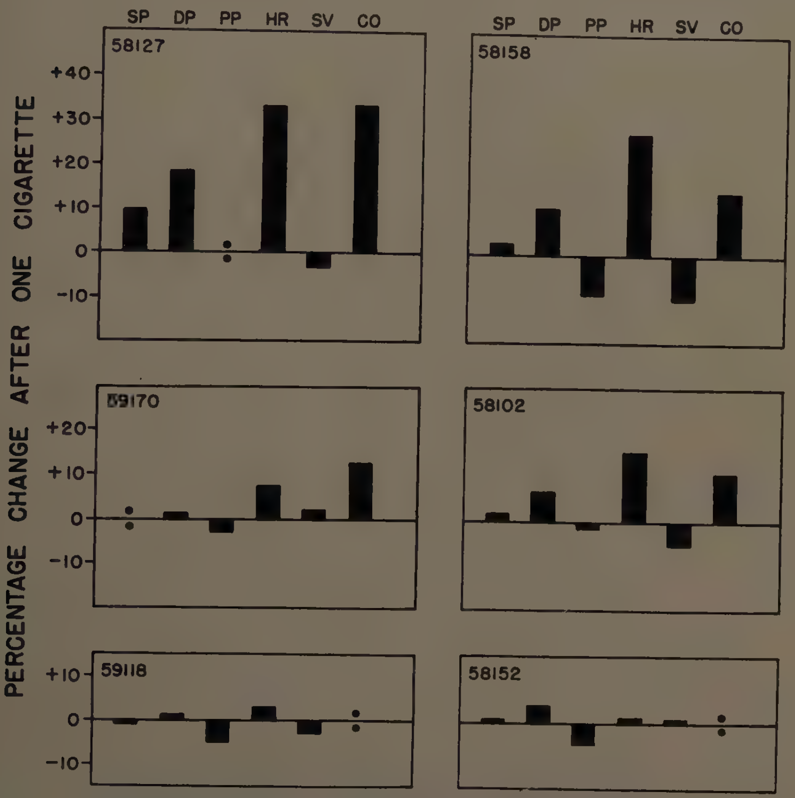

FIGURE 3. The individual circulatory response to smoking. Each column represents the mean of the changes in eight tests. The subjects are arranged in order of reactivity. Reproduced by permission of *The Journal of Chronic Diseases.*[2]

The influences of time of day and casual versus basal conditions were studied in an experiment on another group of 32 male smokers. Four smoking tests were performed on each subject: two tests on each of two days, one before breakfast and one before lunch. Tests A and B on the same day differed in the following respects: for Test A the subject had been fasting overnight, but in Test B fasting extended only for the four hours since breakfast; in Test A he had not smoked for eight hours, while for Test B he was allowed to smoke up to an hour before the test; before Test A he had been inactive most of the previous eight hours, whereas prior to Test B he had been occupied with the usual morning classes. The tests on the second day were carried out as nearly as possible in the same manner as those on the first. The mean responses to smoking for 32 subjects on each of four occasions are shown in FIGURE 5. The

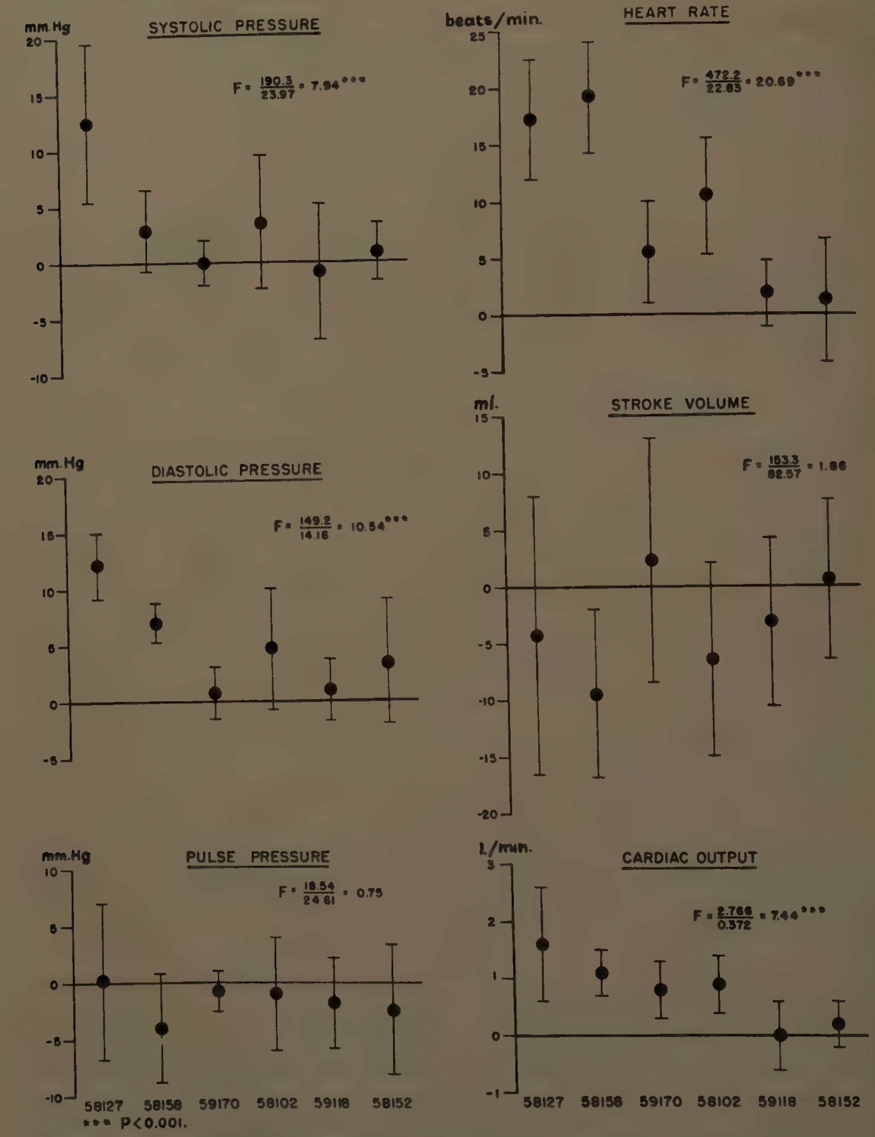

FIGURE 4. Intraindividual variation in the change on smoking in eight tests on each of six subjects. In each instance, the black circle marks the mean value, and the lines, one standard deviation above and below it. F represents the ratio of interindividual to intra-individual variance, and is an indication of the confidence with which subjects may be classified according to the magnitude of the response. Reproduced by permission of *The Journal of Chronic Diseases*.[2]

white bars give the control values, the black bars the values after smoking. Systolic pressure is represented by the top and diastolic pressure by the bottom of the blood pressure bar. In order to show small differences, only the upper portions of the columns for heart rate, stroke volume, and cardiac output are represented, so that changes on smoking appear somewhat exaggerated. It will be seen that the control values for blood pressure, heart rate, and cardiac output were higher on both occasions before lunch than before breakfast. The final figures immediately after smoking were remarkably constant: indeed, they were the most constant readings. These ceiling values varied so little that even if the readings obtained under most unlike circumstances were compared,

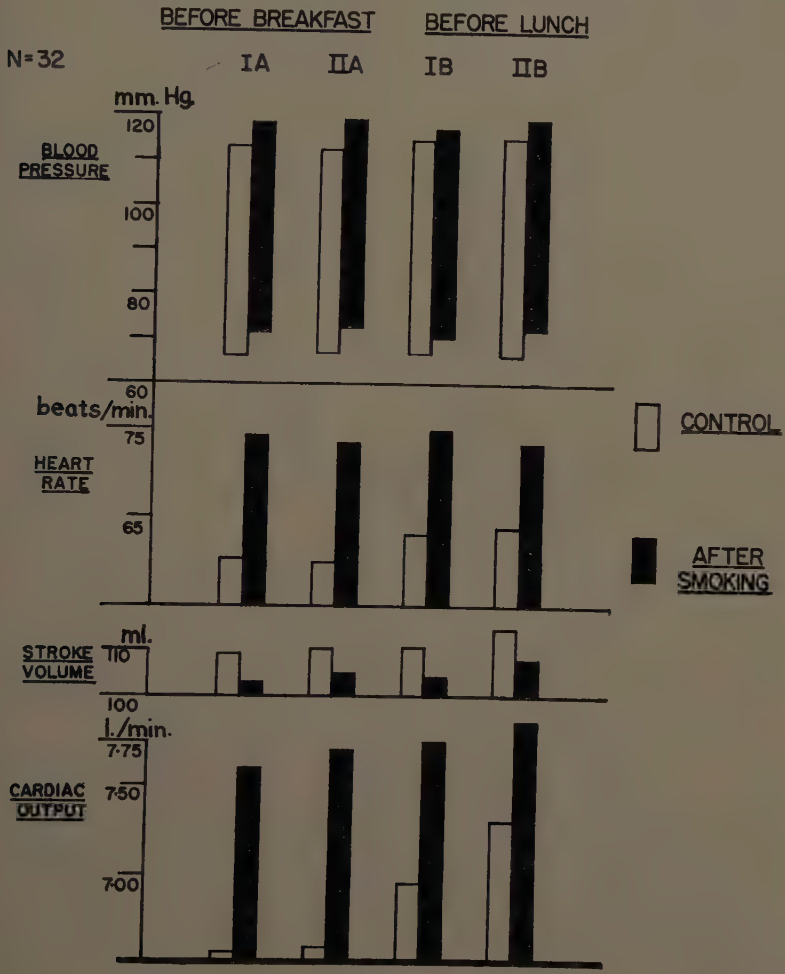

Figure 5. The relative constancy of the final post-smoking values under different circumstances. In order to represent small differences, only the upper portions of the columns for heart rate, stroke volume, and cardiac output are shown. For this reason, changes on smoking appear exaggerated. Reproduced by permission of *The Journal of Chronic Diseases*.[2]

that is, those before breakfast on one day with those before lunch on the other, only two of the 36 combinations were significantly different at the 5 per cent level, about the frequency one might expect by chance. This observation suggests that smoking a cigarette tends to push the various measurements to a ceiling, as if a cigarette at different times of the day "takes up the slack" regardless of the control figure. Moreover, the ceiling is essentially the same for lighter and heavier smokers and differences in habits of exercise did not affect it.

To sum up in regard to reproducibility, comparison of the results obtained in these two series of standardized studies with those carried out originally on male smokers indicates that we gained little by attempting to standardize the conditions of the test beyond those laid down for the original studies. The presence or absence of the basal state appeared to be the only factor of general importance. While slight sharpening of the sensitivity of the test would follow its performance under basal conditions, tests carried out under casual conditions showed satisfactory reproducibility for practical testing purposes.

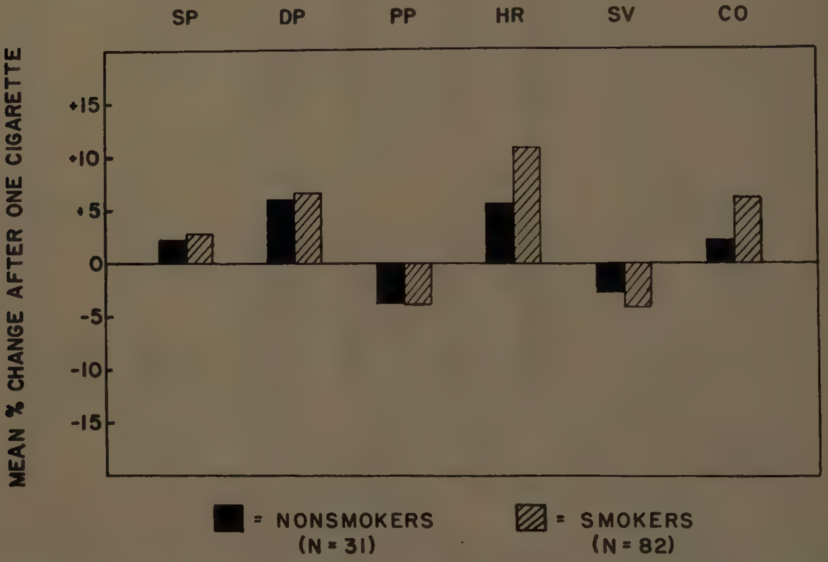

FIGURE 6. The patterns of circulatory response to smoking, comparing smokers with nonsmokers. Reproduced by permission of *Annals of Internal Medicine*.[1]

Let us now consider the meaning of different patterns of circulatory response to smoking. Are they related to other characteristics of the subjects themselves? In our original studies, we investigated the influence of four factors: body weight, sex, smoking habits, and parental history of hypertension or coronary disease.[1] The mean changes after smoking for subjects who were overweight, normal weight, and underweight were almost identical. After smoking a single cigarette, the mean systolic and pulse pressures in a small group of women rose significantly more than in men, but there were no significant differences in the change in diastolic pressure, heart rate, stroke volume, or cardiac output. Comparison of the patterns of subjects with different smoking habits showed that smokers respond to a single cigarette a little more actively than nonsmokers, but these differences did not achieve significance in the preliminary study, where the numbers were small (FIGURE 6). In a later study on larger groups, the heart rate change after smoking was significantly greater than in nonsmokers.[3] Of particular interest was the comparison of the patterns of subjects with different family histories. In FIGURE 7 the pattern of subjects with a parental history of hypertension is compared with the patterns of subjects whose parents are free from cardiovascular disease and of subjects with a history of parental coronary disease. The offspring of hypertensive parents tended to be much more reactive than the offspring of unaffected parents, with a significantly exaggerated increase in cardiac output after smoking one cigarette as well as a greater rise in blood pressure and heart rate, while the offspring of coronary parents showed a significantly more marked diminution in stroke volume and a smaller increase in cardiac output than the offspring of unaffected parents. Accordingly, the difference in response to the smoking test appears to be in part genetically determined, and may be of predictive value in regard to the development of hypertension and coronary disease at an early age, on one hand, or as an index of longevity and freedom from disease on the other.

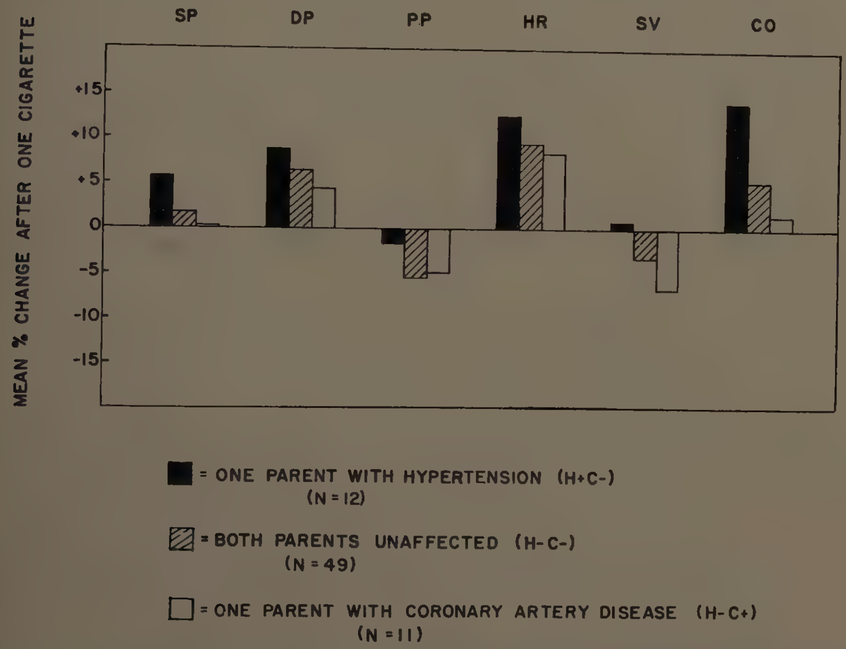

FIGURE 7. The patterns of circulatory response to smoking among subjects with parental history of hypertension, subjects with normal parents and subjects with parental history of coronary artery disease. Reproduced by permission of *Annals of Internal Medicine*.[1]

The finding that subjects with parental hypertension show exaggerated responses to smoking supports the concept that hyperreactivity is a prehypertensive characteristic. Hines and Roth found that patients with clinical hypertension show somewhat greater mean rises in blood pressure after smoking two cigarettes than persons with normal blood pressure, and it is well known that

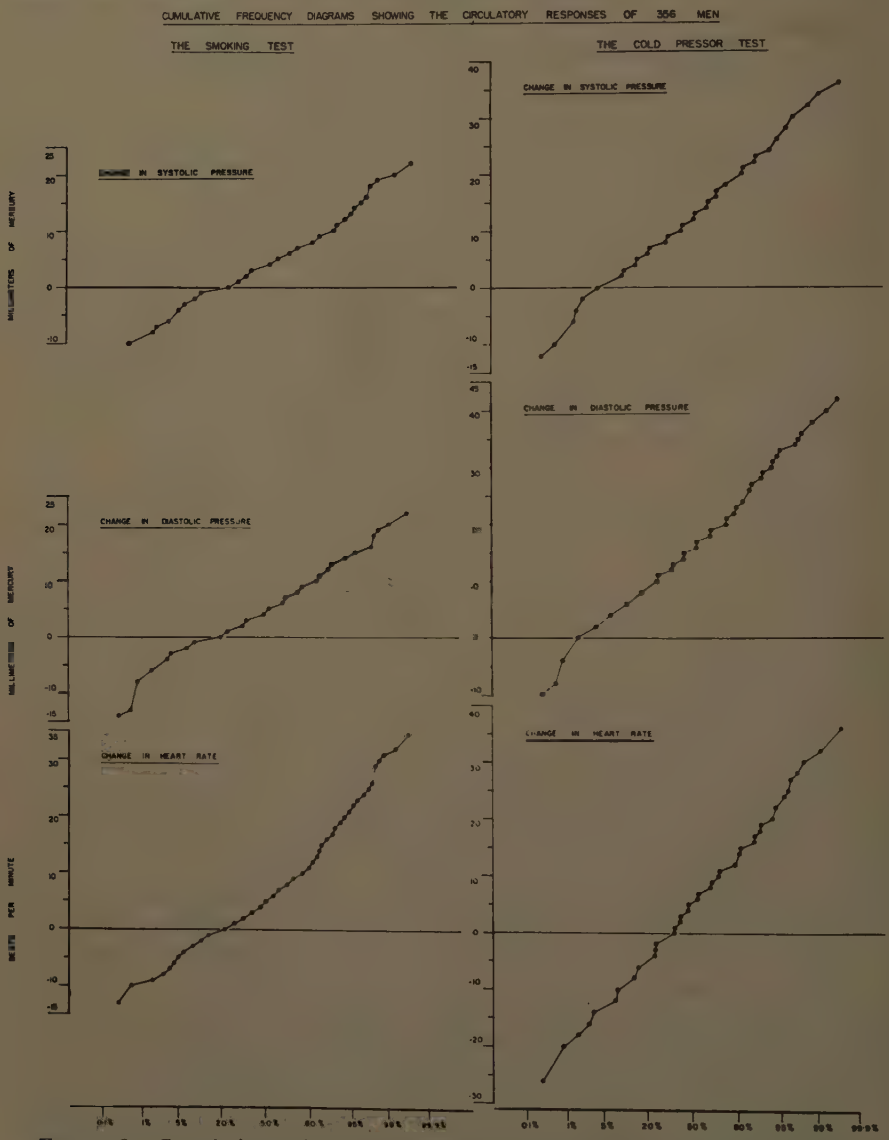

FIGURE 8. Cumulative probability graphs of the three variates measured in each of two tests. Opposite each value is plotted the percentage of all readings that fell at or below this level. Percentages are plotted on a special probability scale that is so constructed that a normal distribution forms a straight line, with the mean lying on the fiftieth percentile, and the standard deviation proportional to the gradient of the curve. Upward convexity of the curve indicates positive skewing, while upward concavity shows negative skewing. Reproduced by permission of *Annals of Internal Medicine.*[3]

hypertensive patients are usually hyperreactors to the cold pressor test of Hines and Brown.[4,5] Roth and her associates, in repeated observations of six subjects, noted limited agreement between hyperreactivity to smoking and to the cold pressor test.[6]

We have compared the responses of blood pressure and heart rate to the smoking test with the responses to the cold pressor test in 356 men. In both tests, age and smoking habits had little influence on the magnitude of the response of blood pressure and heart rate. The distributions of the changes in systolic pressure and diastolic pressure (in mm. of Hg) and in heart rate (in beats per minute) in each of the two tests are presented in cumulative probability graphs in FIGURE 8. Here percentages are plotted on a probability scale so constructed that a normal distribution forms a straight line. It will be seen that each of the three measurements in the two tests does show approximately normal distribution, so that all appear to be continuous variables. Any dividing line or cutting point to distinguish "hyperreactors" in either test, therefore, seems purely arbitrary. Correlations between the responses to the two tests were all relatively low, although some were significant. For example, the systolic rise on smoking showed correlations of 0.18 and 0.19 with the systolic and diastolic response to smoking, respectively; both were significant at the P = <0.001 level. We conclude that independent information is gained from each test. Accordingly, both tests may contribute to the appraisal of individual circulatory reactivity, and one test does not replace the other.

TABLE 1

CHARACTERISTICS OF MALE SUBJECTS WITH AND WITHOUT CHANGES IN BALLISTOCARDIOGRAPHIC FORM BEFORE AND AFTER SMOKING

Characteristic	Changes in BCG form* in percentage	
	Present (18 men)	Absent (227 men)
Age (25 years or over)	50.0	36.6
Overweight (10% or over)	61.1	26.4
Mesomorphy/endomorphy (ponderal index under 13.00)	88.9	52.9
First cholesterol (250 mg./100 cc. or over)	61.1	20.9
Smoker or former smoker	88.9	68.8
Parental history of hypertension and/or coronary heart disease	55.6	32.9

* Significant differences calculated by the X^2 method. Overweight $0.01 > P > 0.001$. Cholesterol $P < 0.001$.

Finally, we have reviewed the records of the 245 students in the classes of 1953 through 1958 who took the smoking test in regard to normality of ballistocardiographic form. Over 98 per cent of the subjects had normal control tracings. One man and one woman showed abnormal form in the control record, while two men showed borderline changes; one of the latter tracings deteriorated further after the first cigarette. After smoking, normal ballistic form became borderline or abnormal in 17 additional records, 15 after the first cigarette and 2 after the second cigarette. In all, 21 subjects (18 men and 3 women), or 7.4 per cent of all those studied, showed borderline or early abnormal ballistocardiograms at the end of the second cigarette.

The borderline changes in ballistocardiographic form consisted in rounding, blunting, or notching of the J and K waves and the occasional presence of early M waves in successive cycles. These changes were more pronounced in most of the records interpreted as abnormal; the small complexes had decreased in size and had become irregular. In three subjects the record was distorted after smoking by a hyperkinetic pattern associated with marked tachycardia.

Certain characteristics of the 18 men with borderline or early abnormalities in ballistocardiographic form are summarized in TABLE 1. Compared with the records of the 227 men with normal form, they show a higher proportion of: (1) older subjects; (2) subjects 10 per cent or more overweight; (3) mesomorphy and/or endomorphy, as indicated by the ponderal index; (4) subjects with higher cholesterol levels: 250 mg./100 cc. or above at first test; (5) smokers and former smokers; and (6) subjects with a positive history of hypertension and/or coronary disease in one or both parents.

The traits characterizing the group with ballistocardiographic changes are those often associated with coronary heart disease, that is, a family history of cardiovascular disease, overweight, mesomorphy and/or endomorphy, and higher cholesterol levels. More than twice as many as expected were overweight, and about three times as many as expected had high cholesterol levels. The mean cholesterol level at first test was 265.0 mg./100 cc. for the 18 men with ballistic form changes, in contrast to a mean of 228.5 mg./100 cc. for 562 men in the classes of 1948 through 1957 (regardless of their ballistocardiographic form).

These studies are exploratory and many questions remain to be answered. We have already progressed enough to learn, however, that the ballistocardiographic smoking test reveals many clues which may prove useful in evaluating individual differences in circulatory homeostatic mechanisms.

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References

1. Thomas, C. B., J. L. Bateman, E. F. Lindberg & H. J. Bornhold. 1956. Observations on the individual effects of smoking on the blood pressure, heart rate, stroke volume and cardiac output of healthy young adults. Ann. Intern. Med. **44:** 874.
2. Thomas, C. B. & E. A. Murphy. 1958. The circulatory response to smoking: the variation in ballistocardiographic smoking tests in healthy young men. J. Chronic Diseases. **8:** 202.
3. Thomas, C. B. & E. A. Murphy. 1959. Observations on some possible precursors of essential hypertension and coronary artery disease: VI. Comparison of the circulatory reactivity to the cold pressor test and to the smoking test. Ann. Intern. Med. **50:** 970.
4. Hines, E. A., Jr. & G. M. Roth. 1938. The effect of tobacco on the blood pressure as measured by a standard smoking test. Proc. Staff Meeting, Mayo Clin. **13:** 524.
5. Hines, E. A., Jr. & G. E. Brown. 1936. The cold-pressor test for measuring reactibility of the blood pressure; data concerning 571 normal and hypertensive subjects. Am. Heart J. **11:** 1.
6. Roth, G. M., J. B. McDonald & C. Sheard. 1944. The effect of smoking cigarettes and intravenous administration of nicotine on the electrocardiogram, basal metabolic rate, cutaneous temperature, blood pressure and pulse rate of normal persons. J. Am. Med. Assoc. **125:** 761.

COMPARISON OF CARDIOVASCULAR AND RELATED CHARACTERISTICS IN HABITUAL SMOKERS AND NONSMOKERS*

Henry Blackburn, Josef Brozek,† Henry L. Taylor, Ancel Keys
Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minn.

There is as yet no convincing theoretical explanation or experimental basis for the apparent statistical association between smoking and higher cardiovascular mortality rates. The voluminous and often conflicting data on acute circulatory effects of smoking lead nevertheless to speculation that cardioaccelerator, pressor, and other responses may precipitate or aggravate manifest cardiovascular disease. However, no evidence exists that there is an acute effect on factors related to pathogenesis, such as atherosclerosis or thrombosis.[1,2] Moreover, there is little available information on the chronic effects of smoking. The interesting studies reported in this monograph have contributed to our knowledge in this regard, but point up particularly the need for more long-term experiments.

One approach to the possible chronic effects of smoking is epidemiological, the comparative analysis of personal characteristics of habitual smokers and nonsmokers. Details of the methodology in sample selection and in securing these data have been, or shortly will be, published elsewhere.[3,4,5] Measurements were made and values are presented for a number of circulatory and related parameters among groups of students, business and professional men, firemen, railroad and rural workers, for a total of 1618 men, ages 17 to 67, in this country and in Finland. Most comparisons are made between the extreme categories of nonsmokers and heavy cigarette smokers. The classification of cigarette smoking intensity was made as follows: Never, never smoked, or occasional and remote history; Occasional, not daily; Light, regular smoker, under 10 cigarettes/day; Moderate, regular smoker, 11 to 20 cigarettes/day; Heavy, regular smoker, 21 or more cigarettes/day; and Stopped, stopped *all* smoking for at least one year.

The relatively few cigar and pipe smokers led us to report data according to cigarette habits alone; where tested, the consideration of multiple smoking habits affected very little either the smoking intensity that had been given for cigarettes or the mean values for particular circulatory items. It should be pointed out that the acute effects of smoking were quite well excluded in the first two groups of highly motivated "guinea pigs" involved in our longitudinal cardiovascular studies at the Laboratory of Physiological Hygiene. They received written and verbal instructions not to smoke before reporting in the morning, in a fasting state for the study. In the field surveys a number of procedures including interviews, X-ray examinations, and clinical examinations were carried out prior to the pulse and blood pressure measurements and during these procedures, smoking was proscribed.

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† Present address: Department of Psychology, Lehigh University, Bethlehem, Pa.

Results

Age. In TABLE 1 are the age comparisons between smoking groups. Where a wide age spread occurred, age-trend analysis for each item of interest was carried out.

Heart rate. In TABLE 2 are the mean basal or resting heart rates for several groups. The basal pulse rate for the first groups and the resting pulse rate for the second are consistently higher in the heavy smokers, reaching statistical significance in some instances; this was corroborated by age-trend analysis.

TABLE 1

AGE IN "NEVER" VERSUS "HEAVY" SMOKERS

	Total No. men		Never	Heavy	$\bar{\Delta}$	Significance
Businessmen	286	Mean	49.2 (67)	48.4 (36)	−0.8	None
		S.D.	3.1	2.9		
Students	159	Mean	20.1 (83)	20.8 (53)	0.7	*
		S.D.	1.9	1.8		
Railroad workers	414	Mean	46.8 (73)	43.4 (138)	−3.4	*
		S.D.	12.1	10.3		
Firemen	234	Mean	39.8 (56)	39.9 (60)	0.1	None
		S.D.	9.5	6.4		

* $P_t < 0.05$.

TABLE 2

BASAL OR RESTING PULSE RATE IN "NEVER" VERSUS "HEAVY" SMOKERS

	Total No. men		Never	Heavy	$\bar{\Delta}$	Significance
Businessmen	286	Mean	64.5 (82)	69.0 (48)	4.5	*
		S.D.	8.4	9.9		
Students	159	Mean	62.7 (83)	65.7 (53)	3.0	†
		S.D.	8.8	7.1		
Railroad workers	414	Mean	68.1 (73)	69.3 (138)	1.2	None
		S.D.	11.9	12.3		
Firemen	234	Mean	72.3 (56)	79.8 (60)	7.5	‡
		S.D.	8.3	12.0		

* $P_t < 0.01$.
† $P_t < 0.05$.
‡ $P_t < 0.001$.

In TABLE 3 are the actual work pulse rates in several groups taken during the course of a 10-min. treadmill walk at 3.5 mph and 5 per cent grade. Although it is not shown in the table, the higher pulse rate in the businessmen smokers was found to be statistically insignificant when individuals with cardiovascular disorders were screened out of the sample and when comparisons were made between nonsmokers and all smokers combined. No significant differences were found in the work pulse increment or in pulse recovery time after effort.

In TABLE 4 are the businessmen's pulse responses to immersion of the hand in ice water and to 6 per cent CO_2 inhalation. No differences were found in comparing smoking categories, nor were any apparent in the young university student group.

Blood pressure. Mean resting auscultatory blood pressures are given in TABLE 5. In the first two groups, of students and businessmen, hypertensive subjects were selected out at the very outset of a long-term follow-up study, now in its thirteenth year. There are no blood pressure differences relating to smoking habit in these two groups, but in examination of the data from broader studies of working populations there is a distinct tendency toward lower systolic

TABLE 3

ACTUAL WORK PULSE RATE IN "NEVER" VERSUS "HEAVY" SMOKERS

	Total no. men	Never	Heavy	$\bar{\Delta}$	Significance
Businessmen	286	114.8 (61)	121.7 (35)	6.9	*
Students	159	118.6 (83)	115.7 (53)	−2.9	None
Railroad workers	414	115.7 (73)	116.6 (138)	0.9	None

* $P_t < 0.05$.

TABLE 4

PULSE RESPONSE TO COLD PRESSOR TEST AND 6 PER CENT CO_2 INHALATION IN "NEVER" VERSUS "HEAVY" SMOKERS

(In Businessmen)

	Never	Heavy	$\bar{\Delta}$	Significance
Cold pressor Pulse increment (mean)	5.2 (67)	6.9 (35)	1.7	None
CO_2 inhalation Pulse increment (mean)	7.4 (67)	9.3 (36)	1.9	None

TABLE 5

MEAN RESTING BLOOD PRESSURES IN "NEVER" VERSUS "HEAVY" SMOKERS

	Total no. men		Never	Heavy	$\bar{\Delta}$	Significance
Businessmen	233	S	123.0 (60)	124.0 (33)	1.0	None
		D	81.1	83.1	2.0	None
Students	156	S	121.3 (83)	120.3 (53)	−1.0	None
		D	68.1	69.4	1.3	None
Railroad workers	414	S	133.8 (73)	129.3 (138)	−4.5	None
		D	80.6	77.5	−3.1	None
Firemen	234	S	135.1 (56)	132.4 (60)	−2.7	Not tested
		D	80.2	76.5	−3.7	
Railroad and firemen combined	648	S	134.4 (129)	130.2 (198)	−4.2	*
		D	79.8	77.2	−2.6	†
Finnish workers	525	S	137.6 (165)	132.3 (360)	−5.3	‡
		D	87.0	84.8	−2.2	*

S = systolic; D = diastolic.
* $P_t < 0.05$.
† P_t approaches 5 per cent level of significance.
‡ $P_t < 0.001$.

and diastolic levels in smokers. This is sometimes statistically significant, as in the combined United States firemen and railroad workers and the Finnish rural workers, and remains significant when the compared groups are rendered homogeneous with regard to age.

Blood pressure responses to the exercise test, a treadmill walk, are given in TABLE 6. There were no differences between the only two groups analyzed thus far, the students and middle-aged businessmen, and there was no difference in blood pressure recovery time.

TABLE 6

MEAN BLOOD PRESSURE RESPONSE TO EXERCISE IN SMOKERS AND NONSMOKERS

	Never	Heavy	$\bar{\Delta}$	Significance
Businessmen				
Systolic increment	5.4 (49)	5.3 (26)	−0.1	None
Diastolic increment	−1.3	0.3	1.6	None
Students				
Systolic increment	16.3 (83)	17.6 (53)	1.3	None
Diastolic increment	−18.8	−17.0	−1.8	None

TABLE 7

MEAN BLOOD PRESSURE RESPONSE TO COLD PRESSOR TEST AND 6 PER CENT CO_2 INHALATION IN "NEVER" VERSUS "HEAVY" SMOKERS

(In Businessmen)

	Never	Heavy	Δ	Significance
Cold pressor				
Systolic increment	9.8 (67)	9.7 (35)	−0.1	None
Diastolic increment	15.8 (82)	11.7 (47)	−4.1	*
CO_2 inhalation				
Systolic increment	16.6 (82)	21.1 (47)	5.0	†
Diastolic increment	9.7 (67)	11.5 (36)	1.8	None

* $P_t < 0.01$.
† $P_t < 0.05$.

Responses by the students in these studies to the cold pressor test are not different, which is similar to the results Thomas and Murphy reported for their young subjects.[6] In TABLE 7, however, middle-aged heavy smokers show a significantly smaller diastolic pressure rise on cold immersion. This difference is exaggerated when the entire sample, including subjects with cardiovascular disease, is considered.

Systolic blood pressure response to CO_2 inhalation is greater in heavy smokers, but the significance of this difference disappeared when the smoking group was enlarged to include all smokers ($N = 112$).

Cardiac output. In TABLE 8 are the mean resting cardiac outputs in a small number of the middle-aged businessmen. These are acetylene method values, uncorrected, and are systematically lower than Fick values. The mean values are similar for cardiac output, but AV oxygen difference is apparently greater in smokers than nonsmokers.

To interpret these isolated circulatory findings better we shall briefly consider some related variables of basal oxygen consumption, relative obesity, and body build.

Basal oxygen consumption. In TABLE 9 basal oxygen consumption is seen to be slightly higher in heavy smokers, so the data for all smokers combined was examined and the oxygen consumption found to be significantly higher in smokers of the businessmen group. This is not seen in the student group.

TABLE 8

RESTING CARDIAC OUTPUT (ACETYLENE METHOD) IN SMOKERS VERSUS NONSMOKERS OF BUSINESSMEN GROUP

	Never (N = 32)	Moderate and Heavy (N = 34)	Δ	Significance
Cardiac output (l./min.) mean	4.8	4.7	−0.1	None
AV oxygen difference (cc./1000 cc.) mean	65.3	69.5	4.2	*

* $P_t < 0.05$.

TABLE 9

BASAL OXYGEN CONSUMPTION (CC./MIN./M²) IN SMOKERS AND NONSMOKERS

	Total no. men		Never (N = 67)	Heavy (N = 36)	$\bar{\Delta}$	Significance
Businessmen	286	Mean S.D.	113.3 11.6	117.9 11.4	4.6	*
				All smokers (N = 114)		
Businessmen	286	Mean S.D.	113.3 11.6	117.7 12.0	4.4	†
Students	159	Mean S.D.	128.4 10.2	128.2 —	−0.2	None

* Approaches 5 per cent level of significance.
† $P_t < 0.05$.

Relative body weight, fatness, and body build. Because pulse and blood pressure and their responses to stress tests are affected by relative obesity it is of interest to examine a few measures of body fatness. In TABLE 10 relative body weight (from standard height-weight tables) is given for these groups. The heavy smokers are consistently less "obese" than nonsmokers and, in the combined worker groups, the difference becomes statistically significant.

In TABLE 11 relative fatness, as measured by a more reliable technique, body densitometry, reveals a similar difference, but it is not statistically significant, nor are the differences in the sum of 3 skin folds in the identical groups. Increasing the numbers by adding "moderate" smokers did not change the significance. The sum of 2 skin folds in the Finnish population is significantly smaller in all smokers combined.

Body build. Body build from skeletal measurements is of interest in such group comparisons, as basic differences in body structure may be reflected that would suggest inherited, rather than environmentally determined, differences between smokers and nonsmokers. In TABLE 12 is given an index of the pelvic and shoulder diameters divided by height; no difference is apparent between smokers and nonsmokers. Moreover, the standing height alone is not different.

TABLE 10

RELATIVE BODY WEIGHT IN SMOKERS AND NONSMOKERS

		Never	Heavy	$\bar{\Delta}$	Significance
Businessmen	Mean	100.3	98.9	−1.4	None
	S.D.	14.8	14.6		
Students	Mean	108.5	104.1	−4.4	*
	S.D.	14.8	14.3		
Railroad workers	Mean	105.9	103.1	−2.8	None
	S.D.	11.5	12.5		
Firemen	Mean	112.0	108.6	−3.4	None
	S.D.	10.8	10.4		
Railroad and firemen combined	Mean	108.5	104.8	−3.7	†
	S.D.	11.6	12.1		

* Approaches 5 per cent level of significance.
† $P_t < 0.01$.

TABLE 11

FATNESS IN "NEVER" VERSUS "HEAVY" SMOKERS OF BUSINESSMEN GROUP

	Never	Heavy	$\bar{\Delta}$	Significance
Sum of 3 skin folds (mm.)	37.6 (65)	33.4 (35)	−4.2	None
Body fat (%)	24.6 (65)	24.2 (35)	−0.4	None

FATNESS IN "NEVER" VERSUS "ALL CURRENT SMOKERS" IN FINNISH WORKERS

	Never	All	$\bar{\Delta}$	Significance
Sum of 2 skin folds (mm.)	21.9 (165)	18.3 (360)	3.6	*

* $P_t < 0.05$.

TABLE 12

ANTHROPOMETRIC INDEX (LATERALITY-LINEARITY) IN SMOKERS AND NONSMOKERS IN BUSINESSMEN GROUP

		Never (N = 64)	All regular smokers (N = 91)	$\bar{\Delta}$	Significance
Bicristal plus biacromial diameter/standing height	Mean	39.4	39.7	0.3	None
	S.D.	1.33	1.50		

Hemoglobin. Eisen and Hammond[7] and others have reported higher hemoglobin values in heavy smokers. The data in our material show a consistent trend toward higher values in smokers, according to intensity of smoking, but the difference is nowhere significant. A direct comparison of Never and combined smokers is given in TABLE 13.

Lipids. Having considered other variables known to be associated with cardiovascular mortality, such as obesity and blood pressure, we shall now examine cross-sectional comparisons of blood lipid values in habitual smokers and nonsmokers. In TABLE 14 a tendency to higher mean cholesterol levels in smokers is found, never significant in the United States groups, but highly significant and occurring in all age groups among the Finnish population. Further breakdown of the United States cholesterol data into smoking categories for groups above and below the median of relative body weight revealed no differences. In TABLE 15, the cholesterol in the beta fraction is not different, but the businessmen smokers are found to have a lower cholesterol/phospholipid ratio.

TABLE 13
HEMOGLOBIN IN SMOKERS AND NONSMOKERS
(Businessmen)

		Never (N = 65)	Moderate and Heavy (N = 73)	$\bar{\Delta}$	Significance
Hemoglobin (gm.%)	Mean S.D.	15.43 0.93	15.69 0.97	0.26	None

TABLE 14
SERUM CHOLESTEROL IN "NEVER" VERSUS "HEAVY" SMOKERS

	Never	Heavy	$\bar{\Delta}$	Significance
Businessmen	234.8 (65)	242.7 (73 M + H)*	7.7	None
Students	173.7 (83)	179.0 (53 M + H)*	5.3	None
Railroad workers	224.8 (73)	232.2 (138)	7.4	None
Firemen	241.7 (54)	251.1 (59)	9.2	None
Finnish workers	222.6 (165)	248.1 (360)	25.5	†

* M + H = Moderate plus Heavy smokers.
† $P_t < 0.001$.

In TABLE 16 a comparison of alimentary lipemia curves, following a standard 90 gm. fat meal among a "coronary" group, reveals no differences at selected time intervals, and none was found between combinations of values representing predominantly "absorptive and clearance phases."

In TABLE 17 the dietary fat consumption of businessmen smokers and nonsmokers is not significantly different. These data represent actual fat analyses from duplicate meal portions saved by the wives of the subjects for a one-week period.

Other metabolic measures. Two other metabolic measures, indirectly related to lipid measures, are blood glucose and protein-bound iodine. These are given for the businessmen, in TABLE 18, and reveal an interesting higher fasting blood sugar (Somogyi method) in smokers, and no difference in plasma iodine.

Respiratory measurements. There is an increasingly large body of information on respiratory function and disease related to smoking. There are, however, few reports from populations of substantial size that make cross-sectional comparisons of pulmonary function data according to smoking habit.[4,8] In

TABLE 15

CHOLESTEROL/PHOSPHOLIPID RATIO AND BETA LIPOPROTEIN CHOLESTEROL IN SMOKERS AND NONSMOKERS OF BUSINESSMEN GROUP

		Never (N = 59)	Heavy (N = 30)	$\bar{\Delta}$	Significance
Cholesterol/phospholipid	Mean S.D.	1.558 0.124	1.460 0.154	−0.098	*
		Never (N = 59)	All current smokers (N = 107)		
Cholesterol/phospholipid	Mean S.D.	1.558 0.124	1.493 0.149	−0.065	*
		Never (N = 62)	All current smokers (N = 95)		
Beta-cholesterol (% of total cholesterol)	Mean S.D.	81.0 5.6	80.6 6.4	−0.4	None

* $P_t < 0.01$.

TABLE 16

ALIMENTARY LIPEMIA IN SMOKERS AND NONSMOKERS: 44 "CORONARY" SUBJECTS

Plasma Optical Density Units at 650 mμ

	Smokers (N = 22)	Nonsmokers (N = 22)	$\bar{\Delta}$	Significance
Fasting	77	59	18	None
2½ hours postmeal	324	338	−14	None
4 hours postmeal	435	466	−31	None
5½ hours postmeal	392	420	−28	None
7 hours postmeal	317	318	−1	None

TABLE 17

DIETARY FAT CONSUMPTION IN SMOKERS AND NONSMOKERS OF BUSINESSMEN GROUP

		Never (N = 33)	All smokers (N = 44)	$\bar{\Delta}$	Significance
Total fat (gm.)	Mean	114.7	116.6	1.9	None
Per cent of fat calories	Mean	40.3	41.3	1.0	None
Animal fat (gm.)	Mean	84.0	86.0	2.0	None
Per cent of animal fat calories	Mean	29.8	30.3	0.5	None

TABLE 19 is the data available on lung compartments from the same businessmen group; age, relative body weight, and acute smoking effects were eliminated in this comparison. The differences are rather small, but there is a smaller vital capacity, larger residual volume, and larger residual volume/total capacity ratio in the smokers. There was also a significantly higher proportion of individuals with elevated RV/TLC ratios (>35 per cent) among the smokers (31.2 per cent compared to 13.8 per cent, P chi square = 0.015).

TABLE 18
FASTING BLOOD SUGAR AND PROTEIN-BOUND IODINE IN SMOKERS VERSUS NONSMOKERS
(Businessmen)

		Never (N = 65)	All current smokers (N = 109)	$\bar{\Delta}$	Significance
FBS (mg.%)	Mean	69.6	74.3	4.7	*
	S.D.	14.9	10.4		
PBI (mg.%)	Mean	5.77	5.52	−0.25	None
	S.D.	1.40	1.15		

* $P_t < 0.01$.

TABLE 19
LUNG VOLUME IN SMOKERS AND NONSMOKERS OF MIDDLE-AGE BUSINESSMEN GROUP*

		Never (N = 65)	All current smokers (N = 125)	$\bar{\Delta}$	Significance
Vital capacity (cc.)	Mean	4320	4109	−211	†
	S.D.	678	644		
Residual volume (cc.)	Mean	1890	2040	150	None
	S.D.	376	608		
Total lung capacity (cc.)	Mean	6228	6170	−38	None
	S.D.	897	905		
RV/TLC (%)	Mean	30.6	33.2	2.6	‡
	S.D.	4.1	6.9		

* N = 221.
† $P_t < 0.05$.
‡ $P_t < 0.01$.

Stopped smoking. It is a common impression that individuals who give up smoking put on weight. We have verified this impression in a previously published study.[9] In TABLE 20 are the average weights of 21 men (E) in the two years after the year in which they stopped smoking, compared to the two years prior to stopping. They stopped under no compunction of illness. A control group (C), matched for body weight at the outset, continued smoking and showed no significant weight change, but the smokers gained an average of 3.73 kg.

We have extended these observations to blood pressure and serum cholesterol changes for the same matched groups, as seen in TABLE 21. There is a significant rise in serum cholesterol in the stopped-smoking group, which is consistent with other studies showing cholesterol rise during periods of positive caloric balance. Some doubt of the significance of this change arises when it is noted that there is a small increase in the mean cholesterol of the control smoking group that eliminates the statistical significance of the group differences. When these longitudinal observations were extended to another subgroup of 21 of the businessmen who had *never* smoked it was found that the average cholesterol rose from 201 to 210 mg. per cent over the same observation period, with no significant change in body weight.

TABLE 20

MEAN WEIGHTS, WEIGHT CHANGES, AND DIFFERENCES IN WEIGHT CHANGE BETWEEN THE 2 GROUPS, CURRENT (C) AND STOPPED (E) SMOKERS*

Group	Experimental (E)	Control (C)
Weight, kg., for years 1 and 2	76.05	76.36
Weight, kg., for years 4 and 5	79.78	75.86
Weight change, mean	3.73	−0.50
S.D.	±1.94	±3.03
Weight change, *t* within group	5.65†	1.18
Weight change difference, kg., (E-C)	4.23	
Weight change difference, *t* between groups	5.39†	

* Reprinted from *Science* by permission of the American Association for the Advancement of Science.[9]

† Statistically highly significant.

TABLE 21

MEAN SERUM CHOLESTEROL AND SYSTOLIC BLOOD PRESSURE, AND THEIR LONGITUDINAL CHANGES IN STOPPED (E) AND CURRENT (C) SMOKERS

Group	Experimental (E)	Control (C)
Cholesterol mg.% for years 1 and 2	221.8	214.6
Cholesterol mg.% for years 4 and 5	232.6	217.2
Change, mean	10.8	2.6
t within group	2.51*	0.52
Change, difference	8.2	
t between groups	1.25	
Systolic BP for years 1 and 2	124.7	122.4
Systolic BP for years 4 and 5	129.0	125.5
Change, mean	4.3	3.1
t within group	1.37	1.15
Change, difference	1.2	
t between groups	0.29	

* $P_t = <0.05$.

Discussion

This approach, essentially statistical, reveals some differences in circulatory and related measurements between men smokers and nonsmokers. However, the differences are small, and certain inconsistencies in the data lead us to be quite circumspect in theorizing about the reasons for these differences. This statistical approach also tends to disguise occasional important individual response patterns to smoking. Thomas' elegant study, in which she has tested cohorts according to familial medical history, modifies to an extent this weakness in approach, and longitudinal studies should provide worthwhile revelations.

It appears that, even upon careful exclusion of acute smoking effect, basal heart rate is faster in smokers than in nonsmokers. Most acute accelerator effects of smoking last under 30 min.,[10] and unmodified nicotine disappears promptly from the circulation.[11] Furthermore, Watts has reported that although pressor amines increase in the circulation after smoking, the total 24-hour excretion is no greater in smokers than in nonsmokers.[12] It may be that the faster heart rate is due to increased metabolic activity, for which other associations found here give supportive evidence. We should prefer that pharmacologists speculate about the chronic effects of smoking on autonomic regulation.

The work and recovery pulse data, in our opinion, provide no evidence for reduced "circulatory fitness" in smokers. When middle-aged subjects with cardiovascular abnormalities were included in the smoking comparisons the actual work pulse was slightly but significantly higher in smokers. This is quite in accord with the safe conventional recommendation that individuals with cardiovascular disease "should not smoke, or should at least cut down." We are looking further into the status of physical training and activity versus sedentary habits in smoking and nonsmoking groups, since this is the most potent factor in determining pulse responses to effort.

The finding of lower resting blood pressure in smokers has been reported in other populations.[13] The fact that this is not apparent in the groups highly preselected for health indicates some interesting possible sources of bias. At the outset, there are no significant differences in the prevalence of smoking habit in many adult male populations we have studied. There is a tendency in our material,[5] as well as in a British report,[13] toward a greater prevalence of hypertension among nonsmokers. Statistical analysis of both these data reveals that the differences are not statistically significant. In the combined United States groups in which blood pressure differences are found after standardization for age, there is also a significantly lower relative body weight in the smokers. If the data are standardized for relative weight, the mean differences in blood pressure between heavy smokers and nonsmokers are no longer significant.

This demonstrates that bias from selection or from other differences in group composition may be operative, and tempers the consideration of direct causal effects of habitual smoking.

In the businessmen group, where no resting blood pressure differences occur, the diastolic pressure response to the cold test is significantly less marked in chronic smokers. Again we desire comment from pharmacologists concerning the possibility of chronic sympathetic blockade, or depletion of vascular tissue norepinephrine by nicotine.

The lipid data are also inconsistent between geographic areas. The higher cholesterol in smokers is clearly significant in the Finnish population at all ages. Gofman *et al.*[14] and Bronte-Stewart[15] have reported this in isolated younger age groupings. E. Orma (personal communication), is considering the interesting possible relationships between smoking, higher plasma thiocyanate levels, reduced thyroid function, and lipid metabolism in the Finnish area where cholesterol differences are found. In our study of middle-aged businessmen there is a low correlation between protein-bound iodine and serum cholesterol,[16] and no cholesterol differences according to smoking habit; accordingly further studies of this relationship are not being pursued. It is of interest that the cholesterol/phospholipid ratio is lower in smokers of that group.

Studies of acute effects of smoking on lipid metabolism reveal no changes in the common lipid fractions following smoking[1] and very questionable effects on alimentary lipemia.[17,18]

From this material and from other unpublished population data it is apparent that smoking cannot be a primary factor affecting cholesterol level. There is a smoking "effect" (statistically) in some populations but not in others, whereas large geographic differences in cholesterol levels are found and cigarette smoking habits are roughly comparable.

The isolated datum on higher fasting blood glucose in smokers, it appears, will simply add fuel to the old conflict from disparate reports of the acute effect of smoking on blood sugar.

The respiratory data are of interest, and subsequent studies more pertinent to ventilatory function are consistent[8] in that there is evidence of impaired function in smokers.

Although the impression is widely held, the evidence that smokers and nonsmokers are fundamentally, genetically, or psychologically different is still meager. Our anthropometric data reveal no differences. The smoking habit appears to cut widely across personality and occupational choice categories. Thomas' contribution is most interesting in that she finds the tendency to smoke and an index of susceptibility to cardiovascular disease to run parallel.[19] Fisher's collection of data on smoking habits in monozygotic versus dizygotic twins suggests inherited differences.[20] These lines of investigation must be pursued if the role of smoking in the pathogenesis of disease is to be clarified.

Other inconsistencies in these data, in regard to the "effect" of the *amount* of smoking, as well as the sources of bias discussed, render dubious the etiologic role of smoking in the observed differences. For these reasons, plus the overall small magnitude of the differences found and the paucity of long-term experimental evidence, we are unable to give serious consideration to these circulatory phenomena as important underlying factors in the increased cardiovascular mortality rate among smokers. It is clear from epidemiological reports[21,22] that the smoking habit is less significantly associated with coronary attack rate than are relative weight, blood pressure, or serum cholesterol. If smoking is the causal factor in excess cardiovascular mortality among smokers it is apparent that it does *not* operate through producing obesity or hypertension, and it is not likely that it significantly affects lipid metabolism. If anything, in fact, smokers are less obese than nonsmokers. This lack of association with variables well known to relate to cardiovascular mortality contributes to argument both for and against a primary role for smoking.

There is no convincing evidence that smoking is involved in the underlying pathogenesis of cardiovascular disease, atherosclerosis, thrombosis, or hypertension. We are left with the ancient impression that the acute pressor and other as yet unknown acute effects of smoking may aggravate extant heart disease or precipitate manifest disease in a latent disease state, and that in this way smoking may exert its principal effect on cardiovascular mortality rate. Berkson[23] has evoked an equally aged concept to explain smoking effects, that of the "rate of living," which Pearl[24] and other biologists have visualized as woven through the fabric of animal life. The suggestion in our data of accelerated metabolism and heart rate should have appeal to this school. Even a Selye response is not inconceivable, which, in turn, suggests another study of smokers and nonsmokers.

References

1. Page, I. H., L. A. Lewis & M. Moinuddin. 1959. Effect of cigarette smoking on serum cholesterol and lipoprotein concentrations. J. Am. Med. Assoc. **171:** 1500.
2. Blackburn, H., E. Orma, G. Härtel & S. Punsar. 1959. Tobacco smoking and blood coagulation. Am. J. Med. Sci. **238:** 448.
3. Karvonen, M., A. Keys, E. Orma, F. Fidanza & J. Brozek. 1959. Cigarette smoking, serum cholesterol, blood pressure, and body fatness. Observations in Finland. Lancet. **1:** 492.
4. Blackburn, H., J. Brozek & H. L. Taylor. 1959. Lung volume in smokers and non-smokers. Ann. Intern. Med. **51:** 68.
5. Blackburn, H., J. Brozek & H. L. Taylor. Pulse rate, blood pressure and circulatory reactivity in smokers and non-smokers. Circulation. In press.
6. Thomas, C. B. & E. A. Murphy. 1959. Observations on some possible precursors of essential hypertension and coronary artery disease. Ann. Intern. Med. **50:** 970.
7. Eisen, M. E. & E. C. Hammond. 1956. The effect of smoking on packed cell volume, red blood cell counts, hemoglobin, and platelet counts. Can. Med. Assoc. J. **75:** 520.
8. Franklin, W. 1958. The effect of smoking on pulmonary function in a working adult population. J. Clin. Invest. **37:** 895.
9. Brozek, J. & A. Keys. 1957. Changes of body weight in normal men who stop smoking cigarettes. Science. **125:** 1203.
10. Hiestund, W. A., H. J. Ramsey & D. M. Hale. 1940. The effect of cigarette smoking on metabolic rate, heart rate, oxygen pulse and breathing rate. J. Lab. Clin. Med. **25:** 1013.
11. Goodman, L. S. & A. Gilman. 1955. The Pharmacological Basis of Therapeutics. 2nd ed. : 622. Macmillan. New York, N. Y.
12. Watts, D. J. & A. D. Bragg. 1956. Effect of smoking on the urinary output of epinephrine and norepinephrine in man. J. Appl. Physiol. **9:** 275.
13. Brown, R. G., T. McKeown & A. G. W. Whitfield. 1957. A note on the association between smoking and disease in men in the seventh decade. Brit. J. Prev. Soc. Med. **11:** 162.
14. Gofman, J. W., F. T. Lindgren, B. Strisower, O. de Lalla, F. Glazier & A. Tamplin. 1955. Cigarette smoking, serum lipoproteins, and coronary heart disease. Geriatrics. **10:** 349.
15. Bronte-Stewart, B. 1956. Smoking and the cardiovascular system. Brit. Med. J. **2:** 659.
16. Tucker, R. G. & A. Keys. 1951. Concentration of serum protein bound iodine in normal men. J. Clin. Invest. **30:** 869.
17. Marder, L., G. H. Becker, B. Maizel & H. Necheles. 1952. Fat absorption and chylomicronemia. Gastroenterology. **70:** 43.
18. Keiser, G. 1954. Statistische Untersuchungen über den Einfluss des Rauchens auf die Angina Pectoris. Cardiologia. **24:** 285.
19. Thomas, C. B. 1958. Familial and epidemiologic aspects of coronary disease and hypertension. J. Chronic Diseases. **7:** 198.
20. Fisher, R. A. 1958. Cancer and smoking. Nature. **182:** 596.
21. Dawber, T. S., F. E. Moore & G. V. Mann. 1959. Some factors associated with the development of coronary heart disease: Six years follow-up experience in the Framingham study. Am. J. Public Health. **49:** 1349.
22. Doyle, J. T., A. S. Heslin, H. E. Hilleboe & P. F. Formel. 1959. Early diagnosis of ischemic heart disease. New Eng. J. Med. **261:** 1096.
23. Berkson, J. 1959. The statistical investigation of smoking and cancer of the lung. Proc. Staff Meeting, Mayo Clin. **34:** 206.
24. Pearl, R. 1928. The Rate of Living; Being an Account of Some Experimental Studies on the Biology of Life Duration. Knopf. New York, N. Y.

Part VII. Effects of Nicotine and Smoking in Cardiovascular Disorders

CARDIAC EFFECTS OF NICOTINE IN THE RABBIT WITH EXPERIMENTAL CORONARY ATHEROSCLEROSIS*

Janet Travell, Seymour H. Rinzler,† Dorothy Karp‡
Department of Pharmacology, Cornell University Medical College, New York, N. Y.

In normal and cholesterol-fed rabbits with coronary atherosclerosis, we observed cardiac effects of nicotine by electrocardiographic studies in the intact animal and by coronary perfusion of the isolated heart.[1] The purpose of the investigation was to determine whether atherosclerotic and normal isolated hearts respond differently to nicotine as they do to ergonovine.[2,3]

METHODS

Groups of male Dutch rabbits about 2 kg. in weight were started contemporaneously either on a stock diet or on the stock diet to which 2 per cent cholesterol, or 2 per cent cholesterol in 6 per cent corn oil, had been added.[3] Serial electrocardiographic ergonovine tests[3,4,5] were performed at monthly intervals to insure that coronary atherosclerosis had developed at the time of coronary perfusion with nicotine. After intravenous ergonovine maleate (0.05 mg./kg.), positivity of the test is indicated by sagging depression of the S-T segment in about 3 to 5 min.

Electrocardiographic effects. In 16 such ergonovine-positive cholesterol-fed rabbits, nicotine was injected intravenously in a dose of 0.05 mg./kg., and the electrocardiogram followed for 30 min. Electrocardiographic effects of nicotine were similarly observed in 12 control rabbits fed the stock diet. Intravenous pentobarbital sodium§ in a dose of 10 to 15 mg./kg. was adequate to keep the animal quiet for electrocardiography if it was not disturbed during this period of observation. For stabilization of heart rate and blood pressure, at least 20 min. was allowed to elapse between intravenous injections of pentobarbital and nicotine.

Perfusion studies. Langendorff coronary perfusion was carried out on 16 ergonovine-positive atherosclerotic and 14 normal isolated hearts. The heart was removed for perfusion under anesthesia with pentobarbital sodium, 15 to 20 mg./kg., intravenously. Single injections of drugs in a volume of 2 cc. were made into the perfusion system to simulate the peak concentration of an intravenous injection in the intact animal. Observations were made for 4 min. afterward, or longer if the effects lasted longer. The modified apparatus and method have been described in detail.[3]

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† Present address: Department of Medicine, The New York Hospital-Cornell Medical Center, New York, N. Y.

‡ Present address: Departments of Psychiatry and Pharmacology, Baylor University, Texas Medical Center, Houston, Texas.

§ Nembutal Sodium Veterinary, Abbott Laboratories, North Chicago, Ill.

Graded doses of nicotine from 0.01 to 0.1 mg. were injected as the bitartrate in a solution freshly prepared from ampoules of aqueous nicotine bitartrate without preservative.* Duplicate tests at each dosage level were done on every heart. Other drugs injected were norepinephrine, ergonovine maleate, nitroglycerin, and purified arginine-vasopressin, free of oxytocin, supplied by Vincent du Vigneaud.[3] Control injections of Ringer-Locke solution were made in every experiment.

Pathological studies. Hearts were sectioned at two levels, stained with hematoxylin and eosin, Masson or Weigert and oil-red-O stains, and examined microscopically. Other cholesterol-fed rabbits were studied for pathological changes, without incidental experimental procedures.

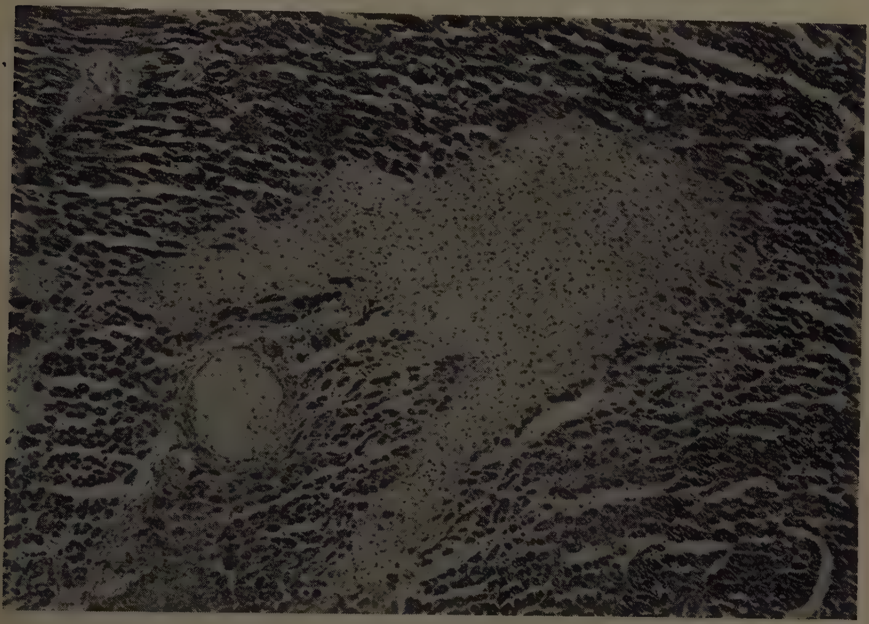

FIGURE 1. Old myocardial infarct and adjacent occluded coronary artery (Masson stain) Rabbit 18 weeks on cholesterol diet.

RESULTS

Cardiac Pathology

Subintimal coronary atherosclerosis was marked in all the ergonovine-positive cholesterol-fed rabbits, whereas it was absent in control animals. Typical pathological changes are illustrated in FIGURES 1, 2, and 3. Old myocardial infarcts and completely occluded small intramural arteries were occasionally seen (FIGURE 1), although the majority of affected arteries were only partially occluded by the subintimal plaques (FIGURE 2). The subintimal elastic membrane showed disintegration at the point where foam cells collected and at the margins of plaques that encroached on the lumen (FIGURE 3). The larger branches of the coronary tree rarely showed lesions that encroached on the lumen.

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* Brewer & Co., Worcester, Mass.

Correlation of pathologic changes in the heart with positivity of the ergonovine test in the cholesterol-fed rabbits used in this and other studies[3,4,5] is shown in TABLE 1. Of 75 animals with a positive ergonovine test, all hearts showed subintimal coronary atherosclerosis on microscopic examination; thus there were no false-positive tests. On the other hand, of 58 animals with advanced coronary atherosclerosis at post-mortem, 26 per cent showed a false-negative result of the final ergonovine test within a few days of sacrifice. The ergonovine test was negative in all control rabbits and microscopic examination of the heart revealed normal coronary vessels; thus, there were also no false-positive tests in this group.

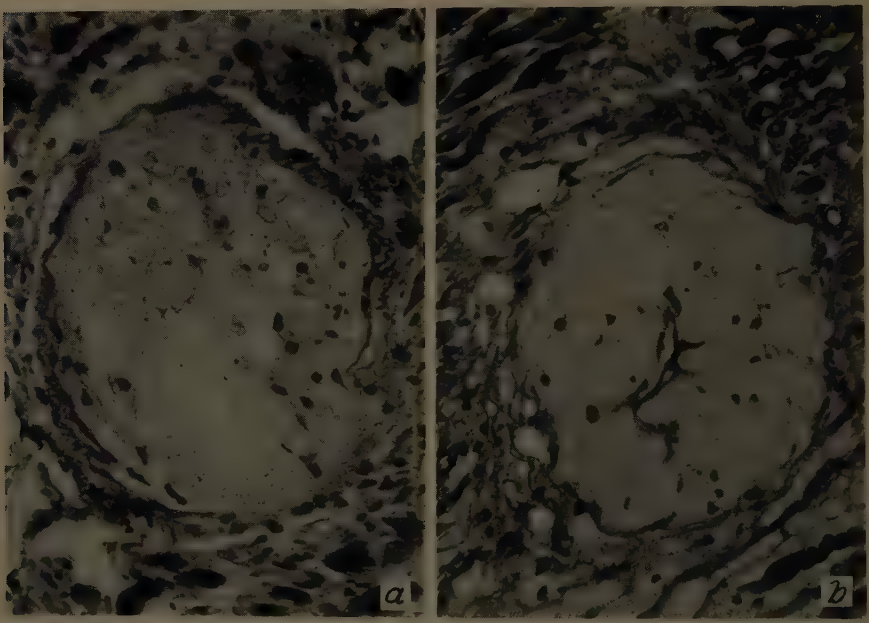

FIGURE 2. Typical subintimal coronary atherosclerosis of (*a*) completely occluded and (*b*) still patent small intramural artery of the cholesterol-fed ergonovine-positive rabbit shown in FIGURE 1 (Masson stain).

Electrocardiographic Effects in the Intact Animal

The small intravenous dose of nicotine caused transient depression of the S-T segment in 2 of the 16 cholesterol-fed ergonovine-positive rabbits, but in none of the 12 normal controls (TABLE 2). In both animals, S-T segment depression was more marked after erognovine than after nicotine. FIGURE 4 shows electrocardiographic records for one of the 2 rabbits with S-T segment

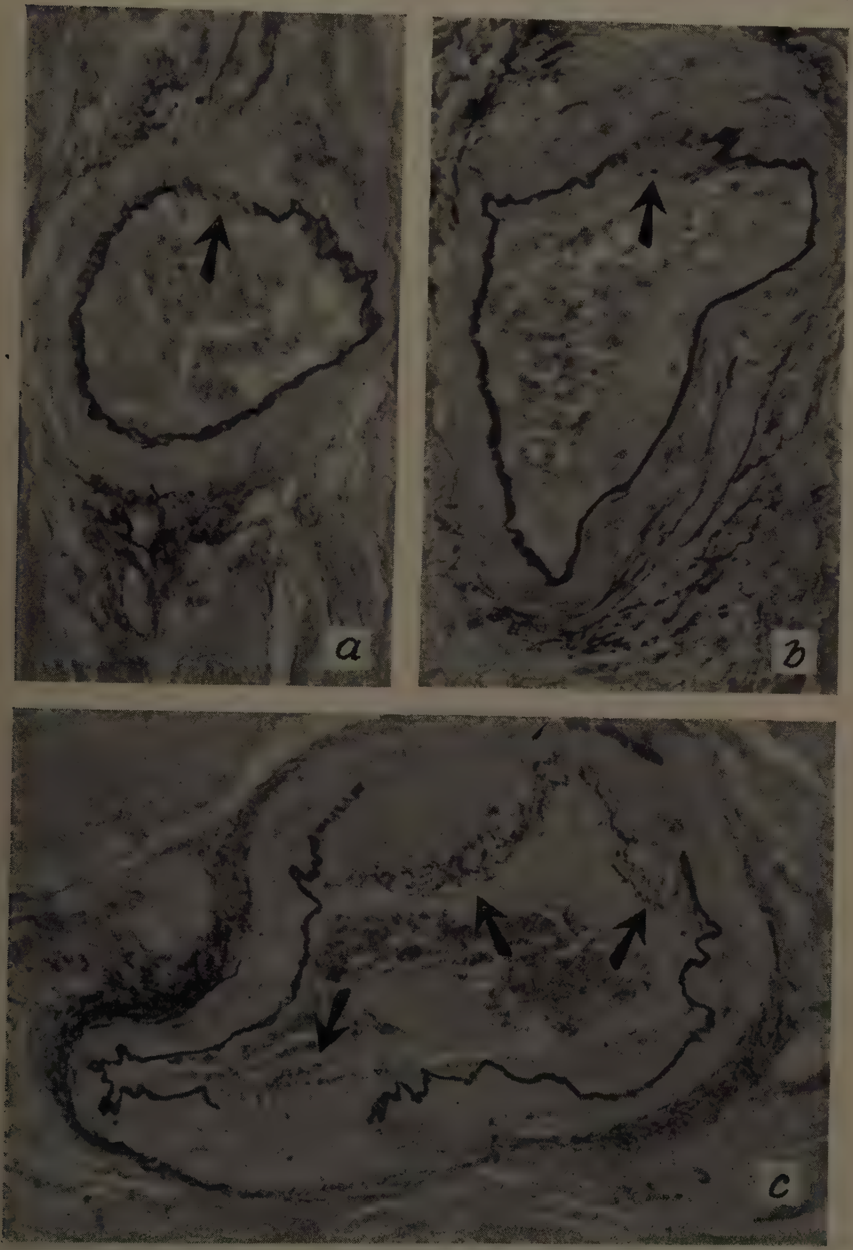

FIGURE 3. Progressive disintegration of subintimal elastic membrane with enlarging atherosclerotic lesions (*at arrows*) in intramural coronary arteries (Weigert stain). Breaks in elastic membrane appear at (*a*) a few subintimal foam cells, at (*b*) a larger collection of foam cells, and at (*c*) margins of plaques that encroach on the lumen.

depression after nicotine, and compares this effect with that observed in the ergonovine test on the preceding day. There was no acceleration of the heart rate to disturb the electrocardiographic interpretation at 3 to 6 min. after injection when the S-T changes were most marked. Depression of the S-T segment had diminished at 20 min., and had disappeared at 30 min. after injection.

TABLE 1

CORRELATION OF POSITIVITY OF FINAL ERGONOVINE TEST WITH SUBINTIMAL CHANGES IN THE CORONARY ARTERIES OF 107 CHOLESTEROL-FED RABBITS

Pathologic changes in coronary arteries	Weeks on diet (av.)	Final ergonovine test	
		Total (No.)	Positive (%)
None	8.0	32	0
Foam cells	9.6	17	18
Plaques*	16.7	58	74

* With encroachment on lumen.

TABLE 2

INFLUENCE OF INTRAVENOUS NICOTINE BITARTRATE ON THE S-T SEGMENT OF THE ELECTROCARDIOGRAM

	Nicotine dose (mg./kg.)	Coronary arteries	Total tests (No.)	S-T segment depression	
				(No.)	(%)
Rabbit	0.05	Normal	12	0	0.0
		Atherosclerotic	16	2	12.5
Man*	0.01	Normal	16	0	0.0
		Atherosclerotic	24	3	12.5

* Boyle *et al.*[6] gave a total dose of 2 mg. nicotine bitartrate (0.6 mg. of nicotine). In calculating the dose per kg., we took the average body weight as 60 kg.

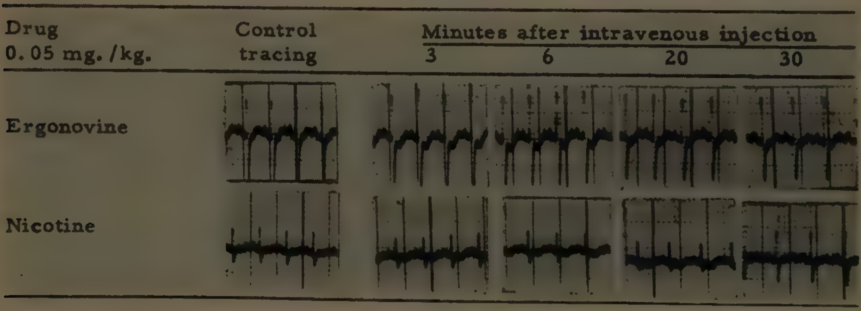

FIGURE 4. Comparison of electrocardiographic changes (lead 2) after intravenous ergonovine and nicotine in cholesterol-fed rabbit with positive ergonovine test. Control heart rate before ergonovine is 312, and before nicotine 305 beats/min. (Sanborn twin-beam electrocardiograph, paper speed 75 mm./sec.). Nembutal anesthesia, 15 mg./kg. intravenously, was administered.

Discussion. After intravenous nicotine the incidence of electrocardiographic changes characteristic of coronary insufficiency in these 16 intact rabbits with coronary atherosclerosis, as shown by a prior positive ergonovine test and subsequent pathologic examination, was 12.5 per cent. The dose of nicotine (0.05 mg./kg.) was of the same order as that employed by Boyle *et al.*[6] intravenously in man (about 0.01 mg./kg.), and these investigators found the incidence of significant electrocardiographic changes was likewise 12.5 per cent in the 24 subjects with coronary heart disease (TABLE 2).

Significance changes in the S-T segment after these small doses of nicotine were not observed in normal groups of either human subjects or rabbits (TABLE 2), or in dogs.[7] Von Ahn,[8] using larger and even fatal doses (1 to 20 mg./kg.) of nicotine intravenously in the intact normal rabbit, likewise did not observe significant changes in the configuration of the electrocardiogram.

Boyle *et al.*[6] concluded that in patients with coronary artery disease the intravenous injection of nicotine may induce a transient state of coronary insufficiency, and our experiments on normal and atherosclerotic intact rabbits are in harmony with this hypothesis. The data yield no information, however, as to the mechanism or site of action of nicotine in producing such electrocardiographic changes. Von Ahn[8] reviewed the variable and conflicting effects of nicotine on coronary flow reported for the isolated heart, heart-lung preparation, and intact animal, and suggested that late coronary constriction after intravenous injection in acute experiments might depend on the release of vasopressin into the circulation, secondary to posterior pituitary stimulation by nicotine.

Effects in the Perfused Isolated Heart

Average effects of graded doses (0.01 to 0.1 mg.) of nicotine on coronary flow, ventricular rate, and contractile amplitude in identical experiments on 6 normal and 7 atherosclerotic hearts are expressed in absolute values in FIGURE 5 and in per cent of the control value in TABLE 3. In FIGURE 6 the same experiments are graphed to show the variability in responsiveness of individual hearts to nicotine with respect to coronary flow. As we have reported,[3] the average basal coronary flow before drugs was higher, the amplitude of contraction was smaller, and the heart rate was slower in the atherosclerotic than in the normal isolated rabbit heart (FIGURE 5). Qualitatively, the effects of nicotine on heart rate and amplitude were the same for both groups, although the positive inotropic effect and acceleration of rate were quantitatively somewhat less in the atherosclerotic hearts. Coronary flow, after an initial reduction, showed a secondary increase in the normal hearts that was absent in these particular atherosclerotic hearts. However, a low incidence of secondary coronary vasodilatation was noted in additional experiments on 9 atherosclerotic hearts in which the same graded doses of nicotine were injected, but in which not every dose was given to each heart. The results for the total series with respect to rate of coronary flow are shown in TABLE 4. For doses of 0.025 and 0.05 mg. of nicotine, 18 of 38 injections (47 per cent) in the normal hearts showed a secondary increase in flow, whereas in the atherosclerotic hearts only 1 of 46 injections (2 per cent) showed such a rise. In the 16 atherosclerotic hearts, 0.1 mg. of nicotine had the same incidence of secondary flow increase as 0.01 mg. had in

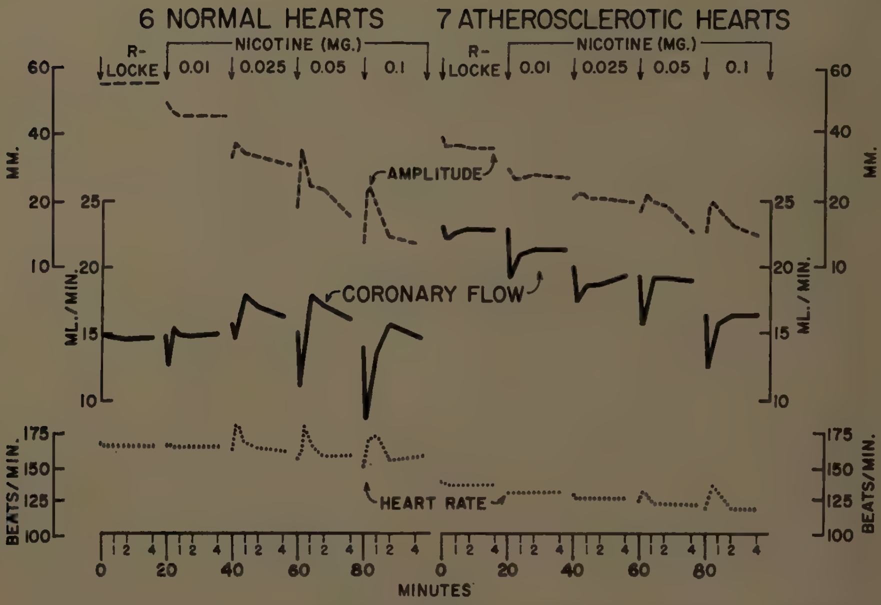

FIGURE 5. Cardiac effects of nicotine in normal and atherosclerotic hearts. Averages are shown for control injections of Ringer-Locke solution (2 ml.) and graded doses of nicotine injected in 2 cc. into the perfusion system close to the heart.

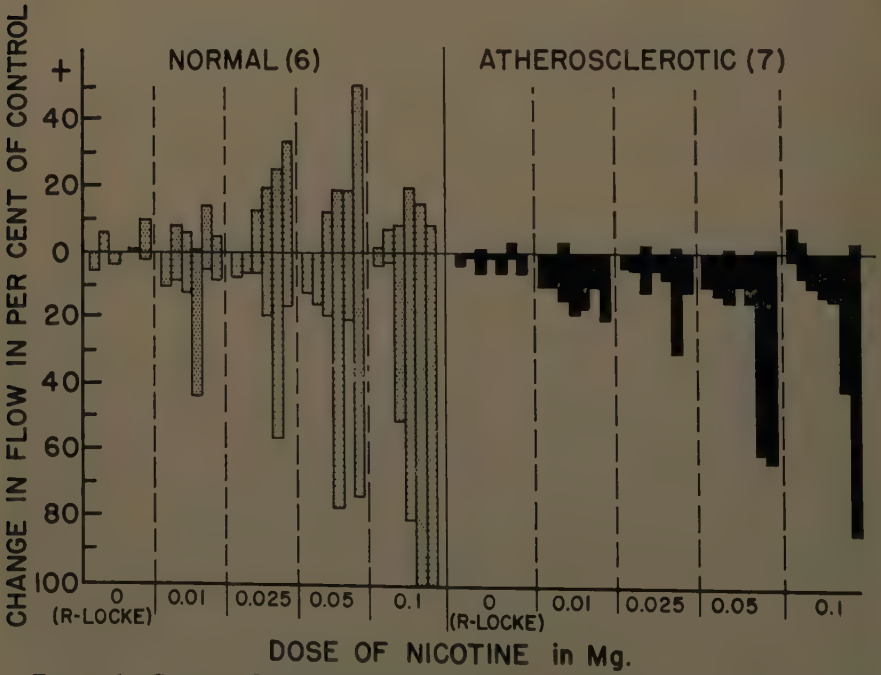

FIGURE 6. Coronary flow in individual rabbit hearts after nicotine and Ringer-Locke solution. The average of duplicate tests with each dose is charted. All decreases in flow (*below baseline*) occurred immediately after injection, and increases in flow (*above baseline*) occurred secondarily. At each dosage, the first bar represents the least responsive heart, the second bar the next in responsiveness, and so forth to the last bar, which shows the most responsive heart.

TABLE 3

COMPARISON OF NICOTINE AND NOREPINEPHRINE EFFECTS ON CORONARY FLOW, HEART RATE, AND CONTRACTILE AMPLITUDE IN PERFUSED RABBIT HEARTS

Hearts (No.)	Drug dose	Coronary flow (% change)		Heart rate (% change)		Amplitude (% change)	
	Nicotine (mg.)	early	late	early	late	early	late
Normal (6)	0 (R-L)*	−2.2	+ 2.8	−2.3	+ 0.3	−1.8	+ 0.8
	0.01	*−14.8*	+ *5.7*†	−2.1	+ 1.1	*−9.3*	+ 0.6
	0.025	*−18.5*	+ *15.3*	−1.6	+ *13.0*	−2.5	+ *13.2*
	0.05	*−36.5*	+ *16.8*	−1.7	+ *16.8*	−1.4	+ *56.8*
	0.1	*−56.7*	+ *10.0*	−0.5	+ *22.6*	−0.9	+ *411.0*
Atherosclerotic (7)	0 (R-L)	−3.6	+ 0.6	−2.3	+ 0.4	−3.4	+ 0.1
	0.01	*−14.0*	+ 0.4	−1.3	+ 1.3	*−9.4*	0.0
	0.025	*−10.6*	+ 0.6	−3.2	+ 1.6	−3.4	+ *10.3*
	0.5	*−26.1*	+ 0.4	−2.9	+ *28.1*	0.0	+ *40.8*
	0.1	*−24.1*	+ 2.0	−1.3	+ *31.4*	0.0	+ *132.2*
	Norepinephrine (µg.)						
Normal (4)	0 (R-L)	−5.6	0.0	−6.1	+ 0.7	−1.1	+ 2.5
	1.0	*−55.7*	+ *27.7*	0.0	+ *38.4*	*−6.8*	+ *146.1*
	2–5	*−40.0*	+ *24.7*	*−17.8*	+ *13.9*	0.0	+ *101.5*
	10	*−60.0*	+ *27.6*	*−11.9*	+ *29.4*	0.0	+ *99.8*
Atherosclerotic (5)	0 (R-L)	−2.6	+ 4.9	−4.2	+ 1.2	−1.5	+ 2.6
	0.5	−0.7	+ 1.3	−2.7	+ 2.3	−2.5	+ *6.4*
	1.0	*−23.6*	+ 3.3	−3.4	+ 2.6	−2.5	+ *29.6*
	2–4	*−56.6*	+ 3.2	−4.1	+ *8.7*	*−7.4*	+ *58.4*

* Control injections of 2 cc. of Ringer-Locke solution.
† Italic figures differ by more than 5 per cent (confidence limit) of the control value.

TABLE 4

INCIDENCE OF SECONDARY RISE IN CORONARY FLOW AFTER NICOTINE

Dose of nicotine (mg./kg.)	14 Normal hearts			16 Atherosclerotic hearts		
	Total injections (No.)	Secondary rise in flow*		Total injections (No.)	Secondary rise in flow*	
		(No.)	(%)		(No.)	(%)
None (R-Locke)	37	2	5.4	38	1	2.6
0.01	17	3	17.7	21	0	0
0.025	21	10	47.6	27	0	0
0.05	17	8	47.0	19	1	5.0
0.1	13	4	30.7	18	3	16.7
All doses	68	25	36.8	85	4	4.1

* An increase greater than 10 per cent of control flow.

the 14 normals, namely, 3 of 18 injections (16.7 per cent) and 3 of 17 injections (17.7 per cent), respectively. Thus, it required 10 times as much nicotine in the atherosclerotic as in the normal heart to produce the same qualitative effect on coronary flow.

In 3 atherosclerotic hearts (FIGURE 7), the effects on coronary flow of graded doses of nicotine were compared with those of ceiling doses of ergonovine,[3] vasopressin, and nitroglycerin given in this sequence. Although hearts differed widely in their general responsiveness to these drugs, in every instance vasopressin was the most potent coronary vasoconstrictor agent and, after this, ergonovine. Nitroglycerin increased coronary flow, although all doses of nicotine had failed to cause secondary coronary vasodilatation in these atherosclerotic hearts.

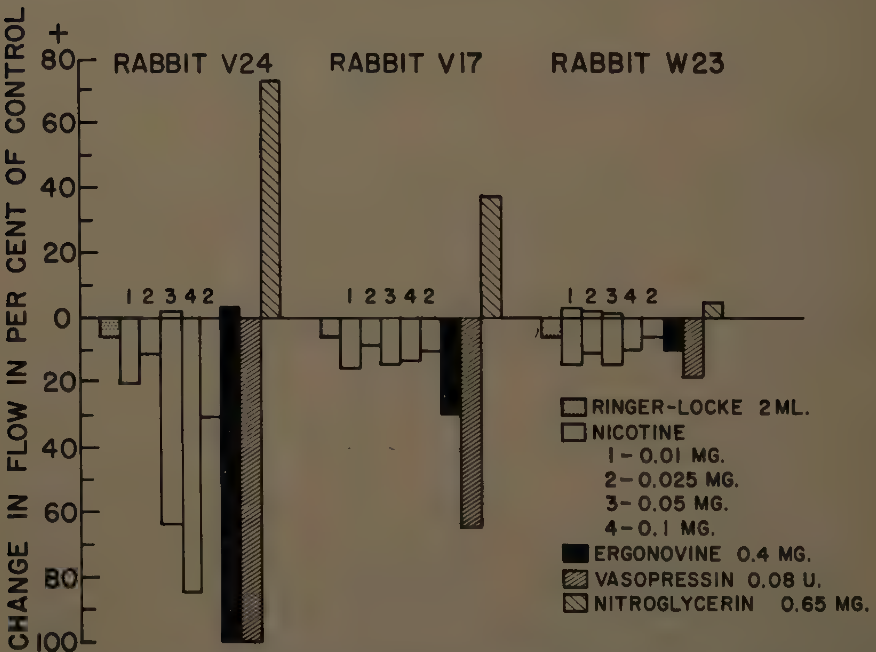

FIGURE 7. Coronary flow in identical experiments on three atherosclerotic hearts. The averages of duplicate tests are charted for Ringer-Locke solution, nicotine, and ergonovine, but only the effects of a single dose in the case of vasopressin and nitroglycerin.

After the graded doses of nicotine, norepinephrine was injected in doses of from 1 to 10 μg. in 4 of the normal hearts and 0.5 to 4 μg. in 5 of the atherosclerotic hearts as shown in TABLE 3. Qualitatively, norepinephrine reproduced the effects of nicotine in both normal and atherosclerotic hearts. In only one respect was there a difference, and that was not statistically significant; in the normal hearts the larger doses of norepinephrine caused a slight initial slowing of heart rate, whereas acceleration was the only change observed after nicotine.

Discussion. Prior to the start of this investigation,[1] studies on the direct action of nicotine on the heart in animals had been carried out only on normals, with one exception: Bellet *et al.*[7] had produced myocardial infarction acutely in dogs by ligating a coronary artery. They have since developed a technique, described elsewhere in this monograph, for inducing chronic coronary insufficiency and myocardial damage more gradually by placing a slowly contracting casein ring on one of the main coronary branches. However, the end result thus achieved does not reproduce the intimal atherosclerosis of the intramural coronary tree that is usually noted in clinical coronary artery disease and in experimental atherosclerosis of the cholesterol-fed rabbit.[9]

A further similarity of the cholesterol-fed rabbit with advanced coronary atherosclerosis to the human subject with the effort angina of coronary insufficiency, but a normal electrocardiogram, is the incidence of positivity of the ergonovine test, namely, 74 per cent and 82 per cent[10] respectively. Wenzel[11] has also found the ergonovine test useful in detecting coronary atherosclerosis in the living cholesterol-fed rabbit, as reported elsewhere in this publication; pathological changes in these arteries develop considerably later than in the aorta and weeks after the peak rise in the serum cholesterol.[5,11]

As cited by Bellet *et al.* in this monograph and by others,[12,13] reported discrepancies in the effect of nitroglycerin on coronary flow may depend in part on the presence or absence of fixed constriction or obstruction of the main coronary branches, because it is the large coronary arteries, rather than the terminal arterial bed, which receive the major impact of ventricular systole and thus largely limit the capacity of the coronary flow to increase.[3,14] Certainly, in the atherosclerotic isolated rabbit heart that failed to show a secondary increase in coronary flow after nicotine (FIGURE 7), the dilator response to nitroglycerin was impressive.

Cardiac effects of nicotine on the isolated hearts of these ergonovine-positive cholesterol-fed rabbits with coronary intimal atherosclerosis differed from those on normal hearts. All differences were quantitative, rather than qualitative. The threshold dose of nicotine that caused an increase in contractile amplitude was similar for both groups, but the absolute increase in amplitude was less for the atherosclerotic hearts. It required twice as much nicotine to produce significant acceleration of heart rate in the diseased as in the healthy heart. With respect to coronary flow, in normal hearts there was quite uniformly an immediate decrease in flow followed in many instances by a secondary increase, whereas in the atherosclerotic hearts a secondary rise in flow after the initial reduction was not observed at the lower dosages. It required ten times as much nicotine in the diseased heart to produce the same incidence of secondary flow increases as in the normals, although the coronary dilator response to nitroglycerin was unimpaired.

Although it is well known that nicotine usually increases coronary flow in normal animals, there is a dearth of studies that compare the effects of nicotine in normal and atherosclerotic hearts. Bellet *et al.*, elsewhere in these pages, found that the dog with chronic myocardial damage and coronary insufficiency due to progressive constriction of a main coronary artery did not show increased coronary flow following nicotine, as did the dog not operated upon; however, the capacity for the coronary flow to increase after nitroglycerin was also lost.

Since it had been shown that in the isolated heart the cardiac accelerator and positive inotropic actions of nicotine are largely mediated by the release of catecholamines,[15,16] mainly norepinephrine,[17] as Burn reports in this monograph, and since it was also known that the intracradiac stores of norepinephrine are depleted in the diseased heart,[18] we compared the cardiac effects of nicotine and norepinephrine in the same normal and atherosclerotic isolated hearts. With respect to all three parameters, qualitatively the cardiac effects of these two drugs were indistinguishable in each group. About 10 μg. of nicotine and 1 μg. of norepinephrine were equivalent. Even the initial reduction in contractile amplitude with the smallest dose of nicotine in normal and atherosclerotic hearts, which might well be considered due to direct stimulation of vagal ganglia, was observed after the smallest doses of norepinephrine in both groups. These findings are in harmony with those of West *et al.*[19] in the normal dog, who found that small doses of nicotine produced only a diminution in myocardial contractility; increases in coronary flow and myocardial contractility seen with larger doses of nicotine were qualitatively similar to those after epinephrine and norepinephrine, and were blocked by tetraethylammonium.

There is little doubt that in the isolated heart the effects of nicotine are mediated by stored adrenergic amines, especially norepinephrine, and that differences in the response of the normal and atherosclerotic isolated heart to nicotine depend on depletion of these intracardiac stores. Since acetylcholine in the atropinized isolated normal heart causes a release of norepinephrine[20] some cholinergic excitatory effects of nicotine on the heart may depend ultimately on local stores of this catecholamine. It has even been suggested[21] that, in the reserpinized isolated heart, nicotine (0.5 mg.) may be injected to assess the degree of catecholamine depletion.

Conclusions

In the isolated heart of the cholesterol-fed rabbit with coronary intimal atherosclerosis and a positive ergonovine test, effects of nicotine differed quantitatively from those in normal hearts. Rate of coronary flow in normals first decreased and then frequently increased; it required ten times as much nicotine in the atherosclerotic heart to induce an equivalent incidence of secondary flow increases. Acceleration of heart rate required about twice as much nicotine in the atherosclerotic as in the normal heart. Contractile amplitude increased with the same threshold dose of nicotine in both groups, but to a lesser extent in the atherosclerotic heart.

Norepinephrine reproduced all the cardiac effects of nicotine when injected in the same normal and atherosclerotic hearts. Roughly, 1 μg. of norepinephrine was equivalent to 10 μg. of nicotine.

Nitroglycerin increased coronary flow in atherosclerotic hearts when nicotine had failed to do so.

Vasopressin (free of oxytocin) and ergonovine decreased coronary flow to a greater extent than did nicotine in the same atherosclerotic hearts.

References

1. Travell, J., D. Karp & S. H. Rinzler. 1957. Nicotine effects on normal and atherosclerotic rabbit hearts. Federation Proc. **16:** 341.

2. KARP, D., J. TRAVELL & S. H. RINZLER. 1956. Ergonovine and vasopression effects on the isolated heart of the rabbit with coronary atherosclerosis. XXth Internat. Physiol. Congr. Brussels, (Abstr. of Communication). : 482.
3. KARP, D., S. H. RINZLER & J. TRAVELL. 1960. Effects of ergometrine (ergonovine) on the isolated atherosclerotic heart of the cholesterol-fed rabbit. Brit. J. Pharmacol. **15:** 444.
4. RINZLER, S. H., J. TRAVELL & D. KARP. 1955. Detection of coronary atherosclerosis in living animal by the ergonovine stress test. Science. **121:** 900.
5. RINZLER, S. H., J. TRAVELL, D. KARP & D. CHARLESON. 1956. Detection of coronary atherosclerosis in the living rabbit by the ergonovine stress test. Am. J. Physiol. **184:** 605.
6. BOYLE, M. N., R. WEGRIA, R. T. CATHCART, J. L. NICKERSON & R. L. LEVY. 1947. Effects of intravenous injection of nicotine on the circulation. Am. Heart J. **34:** 65.
7. BELLET, S., A. KERSHBAUM, R. H. MEADE, JR. & L. SCHWARTZ. 1941. The effects of tobacco smoke and nicotine on the normal heart and in the presence of myocardial damage produced by coronary ligation. Am. J. Med. Sci. **201:** 40.
8. VON AHN, B. 1952. The acute effect of nicotine recorded in electrocardiograms in rabbits. Cardiologia. **20:** 289.
9. RINZLER, S. H. 1957. The Clinical Aspects of Arteriosclerosis. Thomas. Springfield, Ill.
10. STEIN, I., S. H. RINZLER, H. BAKST, J. WEINSTEIN, R. GITTLER & J. TRAVELL. 1954. Cardiac pain in man: Blocking effect of ethyl choloride spray on the angina of ergonovine. Bull. N. Y. Acad. Med. **30:** 318.
11. WENZEL, D. G., J. A. TURNER & D. KISSIL. 1959. Effect of nicotine on cholesterol-induced atherosclerosis in the rabbit. Circulation Research. **7:** 256.
12. BRACHFELD, N., J. BOZER & R. GORLIN. 1959. Action of nitroglycerin on the coronary circulation in normal and in mild cardiac subjects. Circulation. **19:** 697.
13. GORLIN, R., N. BRACHFELD, C. MACLEOD & P. BOPP. 1959. Effect of nitroglycerin on the coronary circulation in patients with coronary artery disease or increased left ventricular work. Circulation. **19:** 705.
14. DUGUID, J. B. & W. B. ROBERTSON. 1957. Mechanical factors in atherosclerosis. Lancet. **1:** 1205.
15. HOFFMANN, F., E. J. HOFFMANN, S. MIDDLETON & J. TALESNIK. 1945. The stimulating effect of acetylcholine on the mammalian heart and the liberation of an epinephrinelike substance by the isolated heart. Am. J. Physiol. **144:** 189.
16. SCHMITTHENNER, J. E., I. FORTE, C. RIEGEL & J. HAFKENSCHIEL. 1956. Nicotine: effects of intravenous infusions on coronary flow, cardiac oxygen and carbohydrate consumption and left ventricular work. Am. J. Physiol. **187:** 630.
17. BURN, J. H. & M. J. RAND. 1958. Action of nicotine on the heart. Brit. Med. J. **1:** 137.
18. RAAB, W. & W. GIGEE. 1955. Norepinephrine and epinephrine content of normal and diseased human hearts. Circulation. **11:** 593.
19. WEST, J. W., S. V. GUZMAN & S. BELLET. 1956. Cardiac effects of intracoronary arterial injections of nicotine. Circulation. **14:** 1017.
20. RICHARDSON, J. A. & E. F. WOODS. 1959. Release of norepinephrine from the isolated heart. Proc. Soc. Exptl. Biol. Med. **100:** 149.
21. MELVILLE, K. I. 1959. Coronary flow and heart action responses following depletion of catecholamines by reserpine. *In* International Symposium on the Catecholamines in Cardiovascular Pathology, held at the Univ. of Vermont, Burlington, Aug. 23–26. Abstr. of Communication. : 13.

THE CHRONIC EFFECTS OF ORALLY ADMINISTERED NICOTINE IN CHOLESTEROL-FED RABBITS*

Duane G. Wenzel, Jasbir Singh Kamal, James A. Turner
School of Pharmacy, The University of Kansas, Lawrence, Kans., and Veterans Administration Hospital, Kansas City, Mo.

The detrimental effects of nicotine on conditions such as Raynaud's syndrome and peripheral arteriosclerosis are apparently related to its vasoconstrictive action. In thromboangiitis obliterans, however, the vasoconstriction produced by nicotine is apparently not the principal adverse effect.[1] Similarly the available evidence concerning the effect of nicotine on the myocardium does not adequately account for the apparent statistical relationship between smoking and the occurrence of coronary artery disease.[2] Attempts to determine directly the chronic cardiovascular toxicity of nicotine with rats and mice have generally been inconclusive.[3] If the relationship of nicotine to these diseases is real, and at least in the case of thromboangiitis obliterans this is true, then its effects must be either more subtle than are likely to be detected by the usual pathological studies with otherwise normal animals, or are the result of an interaction with a concomitant condition. In view of the nature of atherosclerosis and its potential role in the diseases mentioned, it was decided to study the cardiovascular effects of nicotine in the presence of an atherogenic stimulus.

Our initial approach to the problem was to examine the effect of oral nicotine administration upon the serum cholesterol and phospholipid levels of rabbits fed a minimal (0.1 per cent) cholesterol diet.[4] It was found that the daily administration of 2.28 mg./kg. of nicotine to these animals over a 20-week period resulted in significant increases in both plasma cholesterol and phospholipid. Subsequently rabbits were also treated with a more intensive atherogenic stimulus consisting of a 1 per cent cholesterol and 5 per cent oil diet. This was combined with the administration of several dose levels of nicotine.[5] The addition of nicotine to the cholesterol regimen resulted in greater mortality, marked peripheral vascular changes, and electrocardiographic and pathologic evidence of cardiac involvement. The following report describes an attempt to determine the basis for the observed changes.

Experimental

Female albino New Zealand rabbits eight weeks of age and weighing between 2.8 to 3.5 kg. were used in this study. Four groups of 12 animals per group were treated for 24 weeks with the following regimens. Group 1, the untreated control, was fed Purina Rabbit Chow Checkers and water ad libitum. Group 2 received the control treatment plus 1.14 mg./kg./day of nicotine alkaloid in the drinking water. Group 3 was treated by the incorporation of 1 per cent cholesterol and 5 per cent cottonseed oil in the food. Group 4 had the combined treatments of groups 2 and 3. Systolic blood pressure, measured with a Grant ear capsule,[6] was obtained at the onset of the experimental period and at

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four-week-intervals thereafter. The thermal circulation index (TCI), a convenient evaluation of peripheral circulation, was calculated from the following formula:

$$\text{TCI} = \frac{\text{skin temperature} - \text{room temperature}}{\text{rectal temperature} - \text{skin temperature}}[7]$$

Temperatures were obtained with thermistor probes and the TCI values determined at four-week-intervals. The temperature of the skin was measured on the shaved dorsal surface of the lower leg. Coagulation times of the blood (CT) were determined by the capillary method, and were obtained at the initiation of the treatment and at four-week-intervals beginning with the twelfth week.

Results

In a previous study[5] it had been observed that rabbits receiving a hypercholesterolemic diet developed marked lesions of the feet and lower legs. This effect was observed to occur earlier in the course of treatment and with greater severity in those animals that received nicotine in addition to the hypercholesterolemic diet. As nicotine had been reported by others[8] to reduce peripheral skin temperature, it was decided to determine whether the development of the peripheral lesions could be related to the thermal circulation index. The index provided a satisfactory method for the determination of peripheral circulation, especially in chronic studies such as ours in which thermal lag was not an important factor.

From FIGURE 1 it is apparent that the administration of nicotine alone or in combination with cholesterol resulted in a continual reduction in the mean thermal circulation indices during the entire 24 weeks of treatment. The differences between the mean 20-week values of either of the nicotine-treated groups and of their original (zero time) values were significant ($P < 0.001$) when subjected to an analysis of variance.[9] Similarly, the differences between either of the nicotine groups and the control values at the 20-week period were also significant ($0.01 < P < 0.05$). Twenty- rather than 24-week values were employed because of the high mortality in the cholesterol-treated groups during the terminal period of the experiment. While the peripheral lesions were once again observed to occur earlier and to be of greater severity in the nicotine-cholesterol group than in the group receiving the hypercholesterolemic diet alone, there was no significant difference between the mean TCI values of these groups. In other words, if the observed peripheral interaction of nicotine and hypercholesterolemic regimens was related to a decrease in vascularity, this was not apparent from the thermal circulation indices.

It may be seen from FIGURE 2 that the mean systolic blood pressures of all groups increased throughout the entire experimental period. The differences between the mean values of the 0- and 24-week periods were significant for the control ($P < 0.005$) as well as for all other groups ($P < 0.001$). At the 20-week period the mean systolic pressures of all groups differed significantly from one another ($0.001 < P < 0.025$), except for the differences between the nicotine and cholesterol groups and the control and cholesterol groups; these values were not significant. From this information it is apparent that nicotine, in addition to causing anticipated transient changes in blood pressure, induced a chronic cumulative rise in the systolic pressure of rabbits. Furthermore, the combined effect of nicotine administration and a hypercholesterolemic diet resulted in a pressor interaction that may partially explain the increased morbidity and mortality resulting from the combination.

The time-related changes in blood coagulation rate, as illustrated in FIGURE

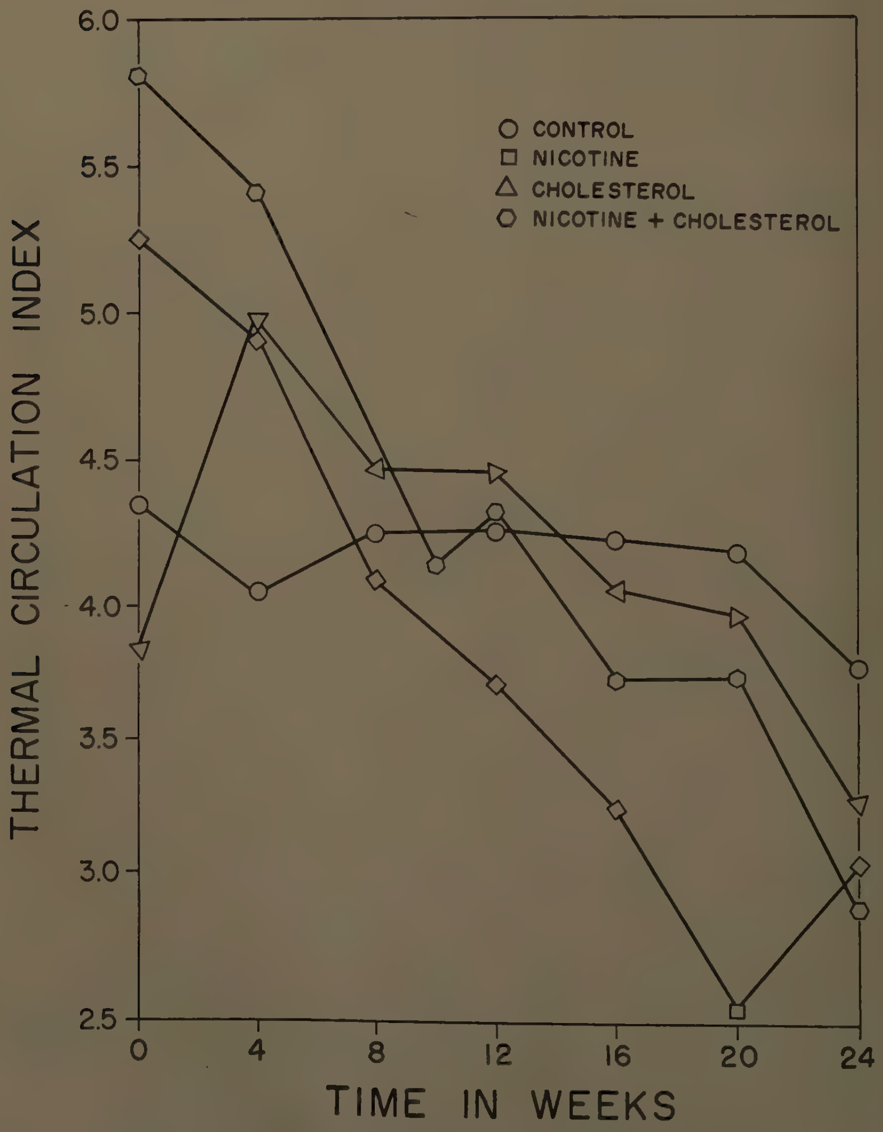

FIGURE 1. Mean thermal circulation indices.

3, indicate that not only does the coagulation time significantly decrease ($P < 0.01$) with age, but that all experimental regimens induced highly significant ($0.001 < P < 0.005$) reductions in the time of coagulation. Furthermore, while all treated groups had greater reductions in the coagulation time at the 20-week period than did the control ($0.001 < P < 0.05$), there were no differences between the treated groups at this time. It may therefore be concluded that under the conditions of this test an interaction between nicotine and the hypercholesterolemic diet upon coagulation time was not apparent.

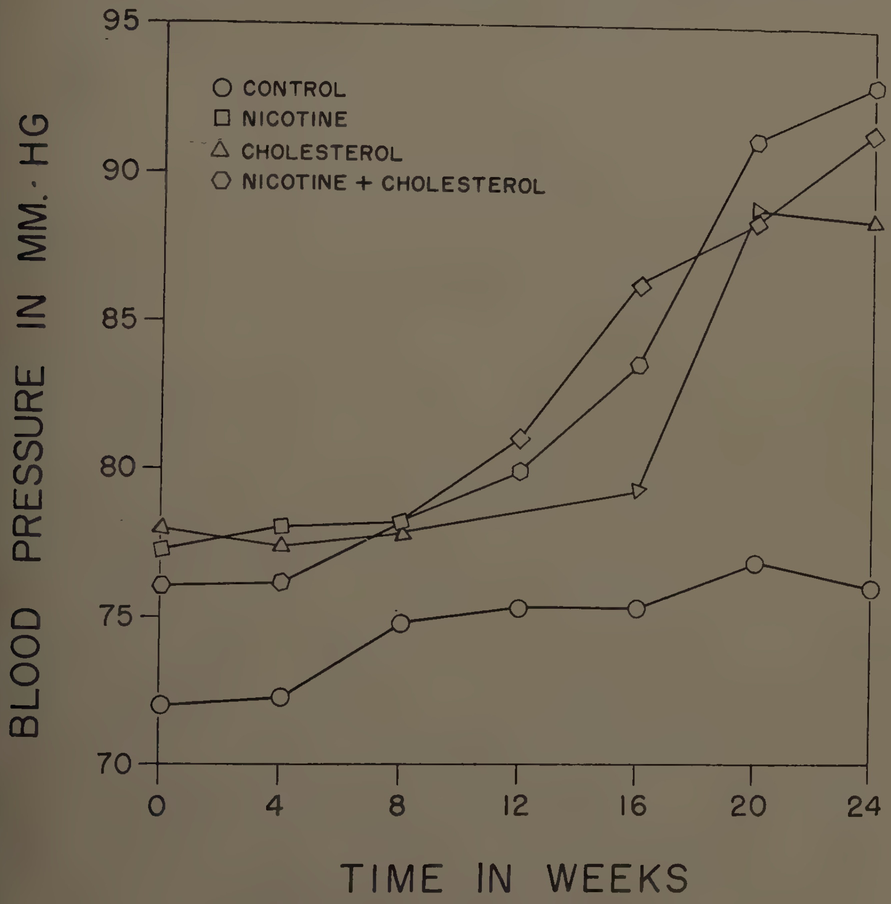

FIGURE 2. Mean systolic blood pressures.

Discussion

From the preceding observations it may be concluded that the chronic administration of nicotine to rabbits, either alone or in combination with a hypercholesterolemic diet, induced hematovascular changes of a character frequently associated with cardiovascular pathology. The elevation of the systolic pressure, reduction of coagulation time, and lowering of the thermal circulation index by nicotine were continuous throughout the 24-week period. Whether any of these effects would have regressed following cessation of the experimental regimens remains to be determined. It seems probable that the changes in coagulation times and the thermal circulation indices were transient effects depending upon the continuation of the treatment. If permanent alterations in the responding system did not occur, the increasing effects with regard to time must reflect either an age-related increase in sensitivity or a cumulative response.

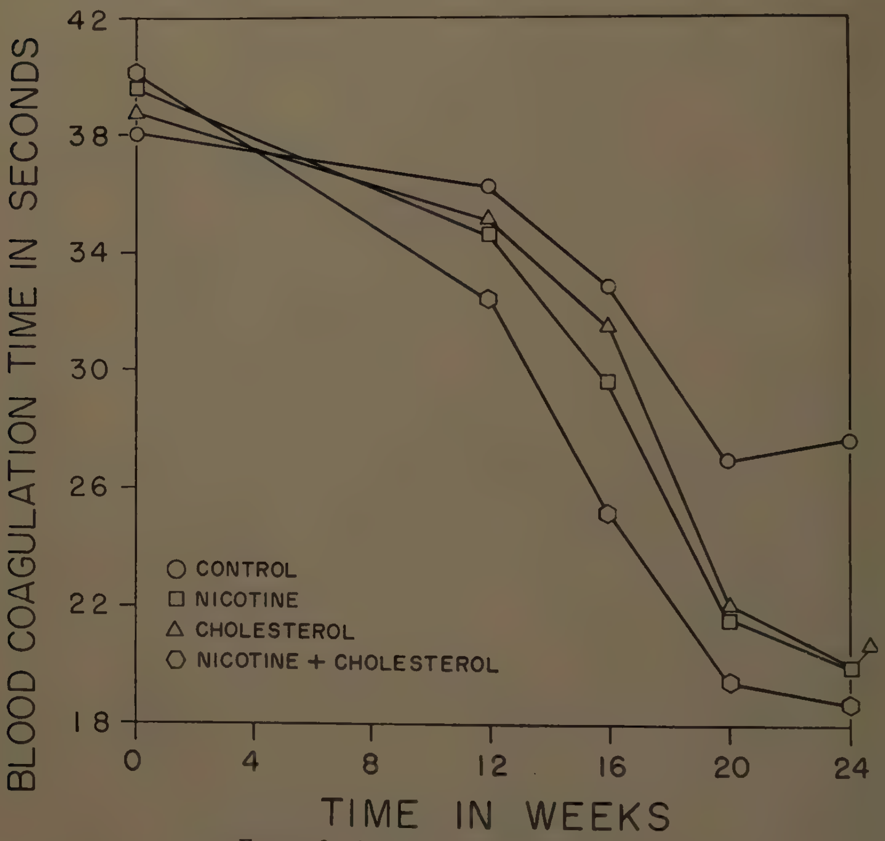

Figure 3. Mean blood coagulation times.

Evidence of an interaction between the nicotine and cholesterol regimens was definite only for the pressor effect. There was no evidence to indicate that the hypercholesterolemic diet decreased the thermal circulation index when administered alone or that it increased the action of nicotine. Inasmuch as both nicotine and the hypercholesterolemic diet resulted in approximately equal reductions in the coagulation time, it is possible that each of these treatments may have individually induced a maximum decrease in the coagulation time which was not further affected by the presence of the other.

References

1. RAPPAPORT, S., H. A. FRANK & T. B. MASSELL. 1950. Circulation. **2:** 850.
2. HAMMOND, E. C. & D. HORN. 1954. J. Am. Med. Assoc. **155:** 1366.
3. THIENES, C. H. & E. M. BUTT. 1938. Am. J. Med. Sci. **195:** 522.
4. WENZEL, D. G. & G. L. BECKLOFF. 1958. J. Am. Pharm. Assoc. Sci. Ed. **47:** 338.
5. WENZEL, D. G., J. A. TURNER & D. KISSIL. 1959. Circulation Research. **7:** 256.
6. GRANT, R. T. & P. ROTHSCHILD. 1934. J. Physiol. **81:** 265.
7. BURTON, A. C. 1934. J. Nutrition. **7:** 497.
8. ROTH, G. M., J. B. MCDONALD & C. SHEARD. 1944. J. Am. Med. Assoc. **125:** 761.
9. SNEDECOR, G. W. 1957. Statistical Methods. : 237. Iowa State College Press. Ames, Iowa.

THE CARDIOVASCULAR EFFECTS OF SMOKING WITH SPECIAL REFERENCE TO HYPERTENSION

Grace M. Roth*
Section of Physiology

Richard M. Shick
Section of Medicine

Mayo Clinic and Mayo Foundation, Rochester, Minn.†

Tobacco long has been suspected of being an etiologic factor in cardiovascular disease, and different types of investigations have been carried out to prove or disprove the relationship. We have been particularly interested in this problem because of our close association with the problems of smoking in peripheral vascular disease and hypertension. Because the man in good health may feel that smoking is bad only for one who is ill, it is important to determine the effect of smoking on normal individuals.

One would expect little difficulty in determining the effect of smoking tobacco on the circulation of normal individuals. However, all methods of measuring peripheral blood flow in man are indirect, and each has its own inherent error. Some of the confusion in the results of smoking tests has arisen because too little attention has been paid to certain factors concerning the status of the individual. The most important of these factors[1] are (1) the environmental temperature, (2) the position of the subject, particularly the extremities, (3) the taking of food and (4) the basal metabolic rate.[2] All must be considered whenever measurements of peripheral blood flow are made. Our standard smoking test, which used the skin temperatures as a measurement of blood flow, took into consideration the four factors just listed and also required simultaneous observations of blood pressure and pulse rates.

Standard Smoking Test

Smoking tests were carried out on normal and other subjects at the Mayo Clinic in a constant-temperature room kept at 25.5° C. (78° F.) and a relative humidity of 40 per cent. The subjects fasted for 15 hours before each test. During the test they wore lightweight short pajamas and were in a supine position on comfortable beds. All unnecessary noise and other stimuli likely to cause vasoconstriction were excluded during the tests. The temperatures of the plantar surfaces of both the first and third toes and the volar sides of the distal phalanges of the first and third fingers were measured at intervals of 10 min. with the copper-constantin thermocouples designed by Sheard.[3] The blood pressure, pulse rates, and basal metabolic rates were determined during the control period. When the skin temperature, blood pressure, and pulse rate were fairly well stabilized, smoking was begun. Cigarettes of different brands bought on the open market were used. The subjects smoked two cigarettes in succession until two thirds of each had been smoked. They inhaled the tobacco smoke with the depth and frequency to which they were accustomed. The blood pressure, pulse rate, and skin temperature were determined simultaneously at intervals of 1 min. during the smoking, which generally lasted 12 to 16 min., and for 30 to 60 min. after smoking had ceased.

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* Present address: Lovelace Clinic, Albuquerque, N. M.

† The Mayo Foundation is a part of the Graduate School of the University of Minnesota, Minneapolis, Minn.

The Effect on the Normal Subject

In order to determine whether the results of smoking tests made on the same individual from day to day are consistent, and whether habitual smokers become so tolerant to tobacco that they do not respond to smoking by vascular or physiologic change, six normal subjects who were habitual smokers and inhaled the smoke were tested. Sixty-six standard smoking tests were carried out on these six subjects, four of whom were physicians and two of whom were women technicians. Their ages ranged from 21 to 44 years, and the basal metabolic rates ranged from −17 to +1 per cent.

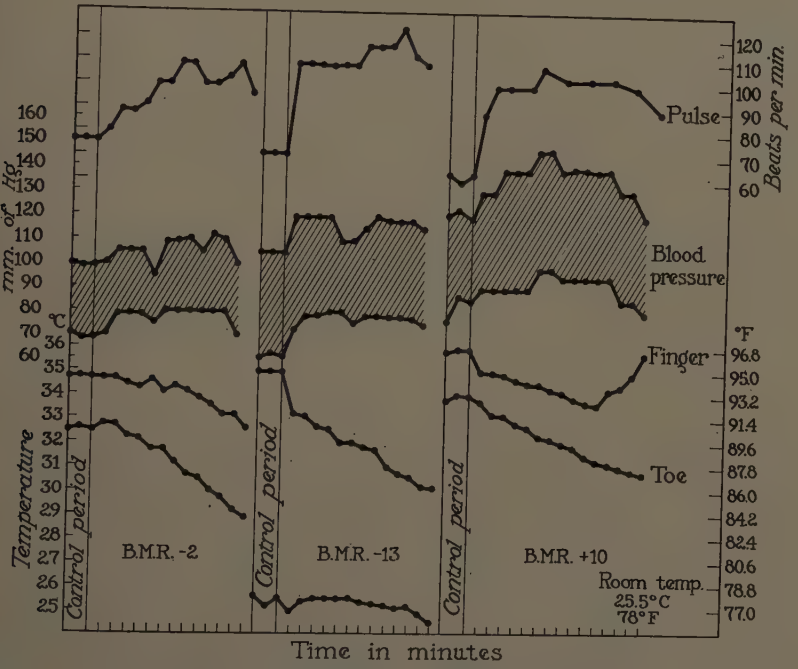

FIGURE 1. Effects of smoking two standard cigarettes on blood pressure, pulse rate, and skin temperature of three normal subjects with various basal metabolic rates.

As controls, cigarettes of corn silk were smoked. The smoking of cigarettes of corn silk caused little or no change in skin temperature, blood pressure, pulse rate, and electrocardiographic tracings.

During the smoking of two standard cigarettes the skin temperature of the extremities of all of the subjects decreased an average of 2.5° C. (4.5° F.) with a range from 1.2° to 6.5° C. (FIGURE 1). During smoking of the two standard cigarettes the average increase of blood pressure was 20 mm. of mercury systolic and 14 mm. diastolic. The pulse rate increased an average of 36 beats/min., the increase ranging from 20 to 52 beats. The electrocardiographic changes consisted of increased heart rate, decreased amplitude of T waves, and even inverted T waves in one subject.

After smoking ceased, the blood pressure, pulse rate, and the electrocardiogram returned to normal within 5 to 15 min. However, the peripheral vascular constriction indicated by the decrease of skin temperatures of the extremities persisted 30 to 60 min., and in some cases much longer.

The skin temperature of the fingers and toes of subjects with variable basal metabolic rates generally decreases not more than an average of 2.5° to 3° C. during the smoking of two standard cigarettes. These 66 standard smoking tests on six subjects showed that the response of the skin temperature of the same individual varied from day to day according to the basal metabolic rate. Thus it was necessary to determine the basal metabolic rate and skin temperature for each study. Increases in blood pressure and pulse rate during smoking varied little from day to day.

Habitual smokers did not show tolerance to the effect of smoking as the skin temperature of the extremities decreased and blood pressure and pulse rate increased during smoking of two standard cigarettes. The decrease of the skin temperature was not related to the length of time the subject had been a smoker or the number of cigarettes smoked daily.

Effect of Nicotine

Additional tests[4] were necessary to determine whether the vascular effects were due to nicotine. When an isotonic solution of sodium chloride was given intravenously to each subject, the skin temperatures of the extremities dropped slightly. When 2 mg. of nicotine was added to the solution without the subject's knowledge, however, the skin temperature decreased rapidly and definitely.

When an isotonic solution of sodium chloride was given intravenously, the amplitude of the T waves in the electrocardiogram was slightly increased. When 2 mg. of nicotine was added to the solution and given through the same apparatus, the increase in heart rate and the decrease in amplitude of the T waves were even greater than those that occurred after the subject had smoked two standard cigarettes. The vascular effects then seem to be due to nicotine.

It was thought advisable to determine whether use of commercial denicotinized cigarettes or cigarettes of low content of nicotine really protects the patient who needs to stop smoking. Accordingly, 30 smoking tests were carried out with various commercially available denicotinized cigarettes. The vascular effects were similar to those of standard cigarettes.

Since no such effect was produced by nicotine-free cigarettes, it seemed desirable to learn how much the content of nicotine in a cigarette should be decreased to banish[1] vascular effects. To do this, 192 standard smoking tests were made on 29 normal subjects who were between 20 and 36 years of age. Six different batches of cigarettes were used. The main-stream of the smoke from one cigarette of each batch contained, respectively, an average of 0.23, 0.55, 1.28, 1.83, 2.47, and 3 mg. of nicotine. As a control, corn-silk cigarettes were smoked. Basal metabolic rates were determined almost every time a smoking test was done. When the cigarettes were smoked, neither the subject nor the technician who was carrying out the test knew which cigarette was being used.

When corn-silk cigarettes and the cigarettes containing 0.23 mg. of nicotine in the stream of smoke were smoked, the skin temperature of the toes changed little, if at all. The skin temperature of the fingers, however, decreased somewhat more when the cigarettes containing 0.23 mg. of nicotine were smoked than when corn silk cigarettes were used. As the concentration of nicotine in the main-stream of cigarettes was increased, the skin temperature of both the fingers and toes decreased more until the effects of the standard cigarettes were found.

The lower the concentrations of nicotine in the smoke, the less the blood pressure and pulse rate increased above the basal level. As the concentration of nicotine increased, both blood pressure and pulse rate increased; the increase was sharp when the concentration of nicotine was raised from 0.55 to 1.28 mg.

Nicotine then has a most important role in production of the vascular changes that accompany smoking, and these observations explain why the same vascular effects were obtained during the smoking of standard cigarettes and denicotinized cigarettes. Apparently the content of nicotine in a cigarette must be decreased more than 60 per cent below that in a standard cigarette before smoking produces only slight or no vascular effects.

Alcohol and Smoking

Alcohol dilates the blood vessels of the extremities, but smoking constricts them. It seemed possible, therefore, that the oral administration of 95 per cent ethyl alcohol would prevent the constriction of the peripheral blood vessels known to be produced by the smoking of tobacco. To test this possibility, observations[5] were made on 65 normal individuals, many of whom were physicians. The ages of the six women and 59 men in this group ranged from 19 to 59 years. The basal metabolic rates ranged from −21 to +5 per cent. Control smoking tests were made, and the next day the effect of smoking after the taking of alcohol was investigated. Both investigations could not be carried out on the same day because the effect of smoking for the control test persisted too long.

Eighty-seven smoking tests were made to determine the effect after alcohol. For this test, the subject took 30 cc. of 95 per cent ethyl alcohol in 250 cc. of fruit juice. This amount is equivalent to 2 fluid ounces (60 cc.) of whiskey and is sufficient to produce definite dilatation of the peripheral blood vessels. The standard smoking test was carried out as soon as an increase of the skin temperature of the toes and fingers indicated vasodilatation.

The blood pressure and pulse rate rose definitely on smoking after ingestion of alcohol in 72 per cent of the subjects, and the rise was only slightly smaller than that during smoking alone. The skin temperature of the fingers and toes, however, decreased below the basal level on smoking after ingestion of alcohol. The height of vasodilatation from alcohol may not be reached until 50 to 60 min. after ingestion of alcohol, but once reached it persists for 60 to 90 min. Smoking tests from 30 to 90 min. after the ingestion of alcohol demonstrated that the alcohol did not prevent vasoconstriction from smoking.

This study then does not substantiate the common belief that drinking a cocktail nullifies the effect of smoking.

From 425 smoking tests with tobacco or corn silk on 100 normal subjects who were habitual smokers, the following observations were made: (1) No tolerance develops to tobacco with respect to the vascular effects; (2) An elevation of the blood pressure and pulse rate and a decrease of the skin temperature of the extremities occur only on smoking tobacco; (3) Nicotine appears to be the most important factor in producing the vascular effects; and (4) Alcohol does not nullify the effect of smoking.

Effect of Smoking on Blood Pressure

In an earlier study with Hines[6] on the effect of smoking on blood pressure in 20 normal subjects with normal reactions to the cold pressor test, the rise in blood pressure during smoking was 17/8 mm. of mercury, while in 10 of those who were hyperreactors it was 30/17. In 56 patients with hypertension as determined by the cold pressor test the rise in the blood pressure was 41/23; in 11 nonsmokers, only 26/9. In the 10 patients with hypertension who had the greatest rise in blood pressure during smoking, the average rise was 47/32 (FIGURE 2).

In order to determine the part played by mechanical and emotional factors in any rise in blood pressure, a control test was made by having the subject go through the same procedure, except that he puffed an unlighted cigarette.

Either the systolic or diastolic blood pressure of all the subjects tested rose somewhat during the smoking test. A considerable rise usually followed the smoking of the first cigarette and a further rise occurred after the second cigarette was smoked. The response of blood pressure to the smoking test was markedly similar to the response to the cold pressor test, except in the group of normal reactors to the cold pressor test. In this group the response of diastolic blood pressure to the two tests was almost identical, but the response of systolic blood pressure to the smoking test was greater in some cases. This may be due to the marked tachycardia that occurred in this group and may be caused by an increase in output of epinephrine, as suggested by Haggard and Greenberg.[7] In the group of normal subjects only those who had an excessive response to the cold pressor test had an excessive response of the systolic and diastolic blood pressures to the smoking test. In all subjects the rise in blood pressure was much less in the control test than on the smoking test, and in some instances the diastolic blood pressure fell during the control test. This was considered evidence that the tobacco smoke was the main factor causing the rise in blood pressure.

In a more recently studied group of normal subjects[8] the rise in blood pressure during smoking was 19/7 from an average basal blood pressure of 105/72. In 60 subjects who were hyperreactors to the cold pressor test the rise was 32/21, whereas in the 10 patients with hypertension the rise was 39/24; in other words, the blood pressure increased from the basal pressure of 198/130 to 237/154. From these studies the effect of smoking tobacco on the blood pressure of the subjects or patients with inherently hyperactive vascular systems, as measured by the cold pressor test, is not due to a nonspecific stimulus acting on a hyperreactive vascular system, but is the result, at least in part, of the nicotine in the tobacco that produces vasoconstriction.

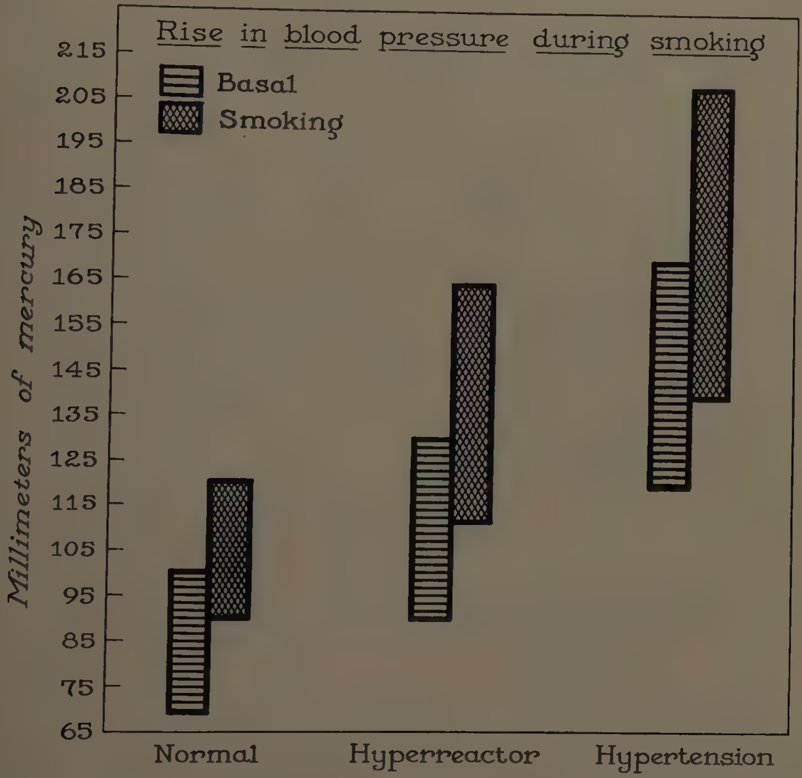

Figure 2. Effect of smoking two standard cigarettes on the blood pressure of a subject with normal reactions to the cold pressor test, of a hyperreactor to the test, and of a subject with hypertension.

The Cause of Vasoconstriction After Smoking

Today evidence is available to support various hypotheses on the cause of the vasoconstriction produced during smoking. That it might be attributed to stimulation of the sympathetic nervous system is demonstrated by the report of Rapaport *et al.*[9] that lumbar sympathectomy abolished the peripheral vasoconstriction produced by smoking in the lower extremities of 19 patients. These authors concluded that the vasoconstriction, therefore, is mediated by sympathetic vasomotor fibers.

We also found[10] (figure 3) no vasoconstriction, as indicated by a decrease in the skin temperature of the toes during smoking, if lumbar sympathectomy was complete, but in this situation the skin temperatures of the fingers would decrease. Thus after lumbar sympathectomy the intact sympathetic nervous system seems to function in a more than adequate manner.

Further work has suggested that this might be a humoral effect, and the studies of Burn and Grewal[11] have shown that nicotine has an antidiuretic action, probably due to stimulation of the hypothalamus with secretion from the posterior lobe of the pituitary, which in turn produces vasoconstriction.

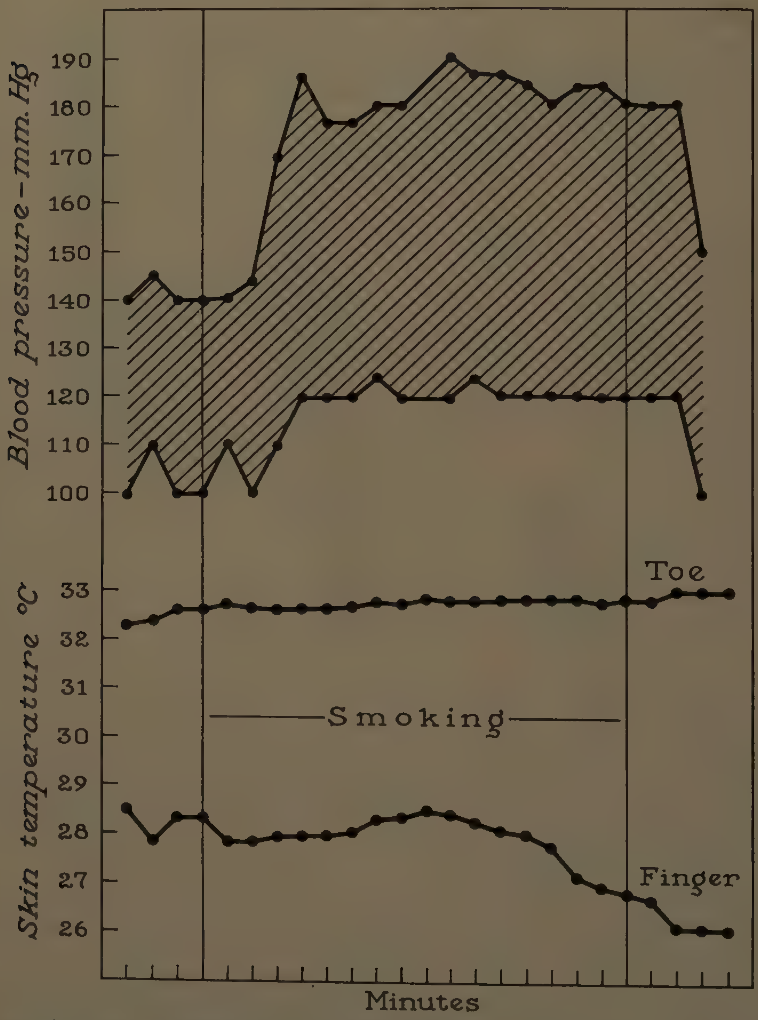

FIGURE 3. Effect of smoking on the fingers and toes after extensive lumbar sympathectomy. Reversal of temperatures of the fingers and toes, with no effect of smoking in the temperatures of the toes but a mild decline in temperatures of the fingers, should be noted. Reproduced by permission of *Diseases of the Chest*.[10]

Recently Burn and Grewal[11] have presented evidence that the stimulant action of nicotine could be attributed to the release of catecholamines, while others suggest that smoking causes increased secretion of epinephrine and norepinephrine.

Schmiterlöw[12] in 1948 showed that norepinephrine is present in the wall of the peripheral arteries. Haimovici[13] reported from perfusion experiments in animals that nicotine may act directly on the blood vessels, because the vasoconstrictor action of nicotine occurred even after the removal of the sympathetic chains and of the spinal nerves.

Similarly Burn and Rand[14] expressed the belief that in rabbits the vasoconstriction is due to the release of norepinephrine from the wall of the arteries.

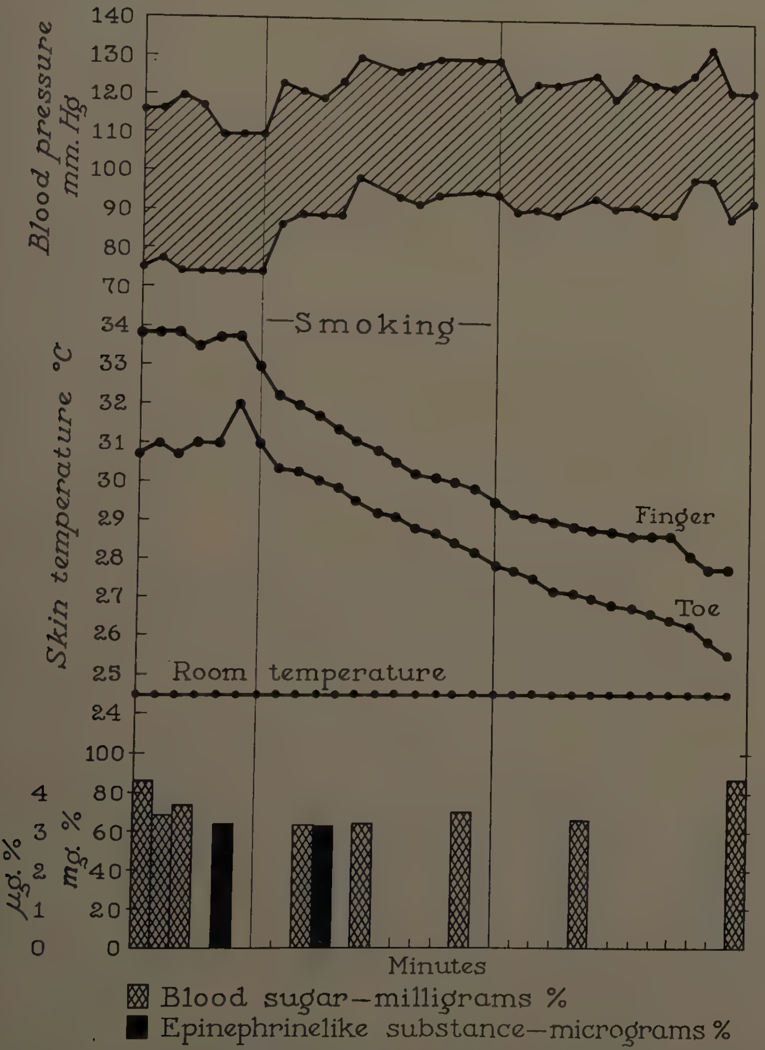

FIGURE 4. The effect of smoking two thirds of two standard cigarettes on the cutaneous temperature of the extremities and on the blood pressure of the same subject. Note the decline in skin temperatures and the rise in blood pressure during smoking and after cessation. Also note the insignificant change in blood sugar and plasma pressor amines before and during the same period of smoking. Reproduced by permission of *Circulation*.[15]

They also observed that in rabbits treated with reserpine, nicotine no longer has a constrictor effect on the perfused vessels of the ear. Furthermore, because of the presence of chromaffin tissue in human skin, Burn and his associates have suggested that thromboangiitis obliterans may be explained by a hypersensitivity to the action of nicotine in releasing catecholamines located near the blood vessels of the skin.

On the other hand, with newer methods available, Rehder and Roth[15] attempted to determine whether smoking of tobacco increased the production of epinephrine and norepinephrine in man and, in turn, increased the blood sugar. Smoking tests on 24 normal subjects were carried out under basal conditions. We found that the levels of the fasting blood sugar and epinephrine-like substances of the systemic blood did not rise appreciably during and immediately after smoking (FIGURE 4). However a definite stimulation of the sympathetic nervous system occurred, as evidenced by the significant rise of the pulse rate and blood pressure and the decrease of the skin temperatures of the fingers and toes. Thus the question still remains whether the vasoconstriction subsequent to smoking and injection of nicotine is related to the amount of nicotine.

References

1. ROTH, G. M. 1951. Tobacco and the cardiovascular system: the effects of smoking and of nicotine on normal persons. *In* American Lecture Series. **100** : 17–32; 40–47. Thomas. Springfield, Ill.
2. ROTH, G. M. & C. SHEARD. 1950. Relation of basal metabolic rate to vasodilatation and vasoconstriction of the extremities of normal subjects as measured by skin temperatures. Circulation. **1:** 1142–1147.
3. SHEARD, C. 1931. The electromotive thermometer: An instrument and a method for measuring intramural, intravenous, superficial and cavity temperatures. Am. J. Clin. Pathol. **1:** 209–226.
4. ROTH, G. M., J. B. MCDONALD & C. SHEARD. 1944. The effect of smoking cigarettes and of intravenous administration of nicotine on the electrocardiogram, basal metabolic rate, cutaneous temperature, blood pressure and pulse rate of normal persons. J. Am. Med. Assoc. **125:** 761–767.
5. ROTH, G. M. & C. SHEARD. 1947. The effect of smoking on the vasodilatation produced by the oral administration of 95 per cent ethyl alcohol or a substantial meal Am. Heart J. **33:** 654–662.
6. HINES, E. A., JR. & G. M. ROTH. 1938. The effect of tobacco on the blood pressure as measured by a standard smoking test. Proc. Staff Meetings, Mayo Clinic. **13:** 524–526.
7. HAGGARD, H. W. & L. A. GREENBERG. 1934. The effects of cigarette smoking upon the blood sugar. Science. **79:** 165–166.
8. ROTH, G. M. & R. M. SHICK. 1955. Cardiovascular effects of smoking of tobacco on normal persons and patients with hypertension. Penn. Med. J. **58:** 281–283.
9. RAPAPORT, S. I., H. A. FRANK & T. B. MASSELL. 1950. The effect of smoking upon blood flow in the sympathectomized limb. Circulation. **2:** 850–858.
10. ROTH, G. M. & R. M. SHICK. 1960. The effects of smoking on the peripheral circulation. Diseases of Chest. **37:** 203–210.
11. BURN, G. P. & R. S. GREWAL. 1951. The antidiuretic response to and excretion of pituitary (posterior lobe) extract in man, with reference to the action of nicotine. Brit. J. Pharmacol. **6:** 471–482.
12. SCHMITERLÖW, C. G. 1948. The nature and occurrence of pressor and depressor substances in extracts from blood vessels. Acta Physiol. Scand. **16**(Suppl. 56): 1–113.
13. HAIMOVICI, H. 1948. Postganglionic site of action of nicotine with special reference to its direct action on blood vessels. Proc. Soc. Exptl. Biol. Med. **68:** 516–520.
14. BURN, J. H. & M. J. RAND. 1958. Noradrenaline in artery walls and its dispersal by reserpine. Brit. Med. J. **1:** 903–908.
15. REHDER, K. & G. M. ROTH. 1959. Effect of smoking on the fasting blood sugar and pressor amines. Circulation. **20:** 224–228.

EFFECT OF NICOTINE ON THE CORONARY BLOOD FLOW IN THE PRESENCE OF CORONARY INSUFFICIENCY: AN EXPERIMENTAL STUDY IN DOGS*

Samuel Bellet, James W. West, Ugo C. Manzoli, Otto F. Muller, Paolo Rossi
Division of Cardiology, Philadelphia General Hospital, and the Department of Pharmacology and the Robinette Foundation of the University of Pennsylvania, Philadelphia, Pa.

It has been shown that nicotine and tobacco smoke in the normal dog and in the human increase the coronary blood flow.[1-3] This is associated with an increase in oxygen consumption and an increase in cardiac work, which can be readily accomplished in the normal heart with normal coronary arteries. The problem that requires investigation is the determination of the effect of nicotine in the presence of various types and degrees of coronary insufficiency, for example, in narrowing of the lumen of the coronary arteries or in the presence of coronary artery disease. This point would appear to be of considerable theoretical and practical importance because many patients over the age of 45 or 50 years with varying degrees of coronary disease and/or coronary occlusion are subjected to the effects of nicotine in tobacco smoke. To meet the demand of increased cardiac work, the coronary blood flow must increase. However, there is little data available relative to the response of the coronary blood flow in the presence of coronary artery narrowing and/or myocardial impairment.

Methods and Material

The following aspects of the problem were investigated in the manner described below:

(1) The effect of nicotine and tobacco smoke on the electrocardiogram of the normal dog was compared with that of dogs at various periods following coronary artery ligation, which corresponded with the acute, subacute, and healed stages of myocardial infarction and after coronary artery narrowing produced by casein rings.

(2) The effect of nicotine on the coronary blood flow was determined in a normal control group and compared with various categories of the group with myocardial infarction.

(3) The effect of varying degrees of coronary artery narrowing was studied by placing casein rings around the circumflex and/or anterior descending branch of the left coronary artery. The casein swells; within a period of 2 or 3 weeks, the lumen is considerably narrowed and, in some instances, almost obliterated. The physiological effects of coronary insufficiency thus produced in dogs resembled to a large degree the effects observed in the human subject.

Coronary blood flow was determined by directly measuring the outflow from the coronary sinus and, in some experiments, by the nitrous oxide method. Catheterization of the coronary sinus was accomplished in the intact animal with a special coronary sinus catheter (modified Morawitz cannula) inserted via the external jugular vein under fluoroscopic guidance, as previously described. The catheter was provided with multiple openings at the tip and an inflatable balloon for securing it in the coronary sinus.

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Methods of coronary blood flow measurement. One-half hour prior to the experiment the animals were given morphine sulfate (3 mg./kg.) intramuscularly and, at the beginning of the experiment, they were anesthetized with a combination of sodium pentothal and Dial urethane (0.25 mg./kg.), given by intravenous injection. One femoral artery was used for direct continuous recording of arterial blood pressures via a strain-gauge manometer connected to a polyviso electrocardiograph; the other was used for intermittent blood sampling. Nicotine was infused into one femoral vein; the other femoral vein was utilized for returning the blood from the coronary sinus cannula to the circulation, which was accomplished by a Dale-Schuster pump. Control measurements were obtained until the blood flow was stabilized. Nicotine was infused at the rate of 20 γ/kg./min. intravenously and coronary flow was again measured continually during a period of 15 to 20 min.

The following data were then obtained: coronary blood flow, O_2 consumption, cardiac output, cardiac work, O_2 extraction, heart rate, and blood pressure. These studies were repeated during and at various intervals for a period of 30 min. after nicotine infusion. At the end of the experiment, the animal was sacrificed and carefully examined to determine the degree of coronary artery narrowing and the state of the heart muscle. In most instances the coronary arteries were injected with Cardiographin and X rays were taken of the injected specimen.

Results

Electrocardiographic observations. It was found that normal unanesthetized animals were able to tolerate a fairly wide range of nicotine (0.2 to 0.8 mg./kg., intramuscularly) with the production of only slight electrocardiographic changes. Following myocardial damage produced by coronary ligation, marked electrocardiographic changes were obtained with a dose that was one-fourth of that required to produce only slight changes in the normal animal. These changes became less marked as the subacute stage was reached, but were still less evident in the chronic stage of infarction. However, the electrocardiographic changes after equivalent doses were more marked in the stage of chronic infarction than in the normal controls* (TABLE 1).

Following the injection of nicotine in dogs with coronary insufficiency (constriction due to casein rings) and after coronary occlusion, there resulted an elevation of the RT segments (FIGURES 1 and 2). These alterations in the electrocardiogram are believed to be due to a discrepancy between the increase in cardiac demand made by the nicotine or the tobacco smoke and the ability of coronary blood flow to increase. That this hypothesis appears likely is indicated by the findings on coronary blood flow given below.

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* The following electrocardiographic changes were classified as slight: alteration in the direction of the T wave, sinoauricular heart block, sinus bradycardia, ventricular escape, simple tachycardia, and occasional ventricular extrasystoles. Moderate changes observed at a more advanced stage of the intoxication consisted of RT deviation, numerous extrasystoles, coupled rhythm, and AV dissociation with a fairly rapid ventricular rate. Marked electrocardiographic changes were observed at a still later period of intoxication and showed nodal tachycardia or ventricular tachycardia at a rate of 220 to 300/min.[4]

Coronary Blood Flow

Normals. The coronary blood flow was determined by the direct method, as described above, in 5 dogs weighing from 20 to 25 kg. The control coronary blood flows ranged between 70 and 140 cc./min. During the infusion of nicotine there was a rapid increase of flow between the first and fifth minute, describing a high early peak with an increase as high as ninefold. The curves of all these dogs showed similar patterns. After the peak the flow decreased rapidly and leveled off to a value higher than the control (approximately 50 to 80 cc./min. higher), where it remained for the rest of the experiment, approximately 15 min. (FIGURE 3).

TABLE 1

SUMMARY OF EXPERIMENTS SHOWING EFFECTS OF NICOTINE ON NORMAL DOGS AND DOGS WITH MYOCARDIAL INFARCTION[4]

	ECG effects	Nicotine dose per kilogram (intramuscular)							
		0.2 mg.		0.4 mg.		0.6 mg.		0.8 mg.	
		No. exp.	Exp. in %	No. exp.	Exp. in %	No. exp.	Exp. in %	No. exp.	Exp. in %
Normal dogs	Marked	0	0	0	0	0	0	2	18
	Moderate	0	0	0	0	0	0	1	9
	Slight	7	100	9	100	7	100	8	73
Infarcted dogs									
Acute (1–4 days)	Marked	13	68	11	84				
	Moderate	6	32	2	16				
	Slight	0	0	0	0				
Subacute (5–11 days)	Marked	2	33	4	33	4	66	1	100
	Moderate	2	33	3	25	1	17	0	0
	Slight	2	33	5	42	1	17	0	0
Chronic (over 18 days)	Marked	0	0	2	17	1	13	5	28
	Moderate	0	0	2	17	1	13	9	50
	Slight	2	100	8	66	5	72	4	22

The cardiac output and cardiac work increased significantly. The coronary AV difference decreased and the oxygen consumption of the myocardium showed an increase.

Coronary artery ligation. This group consisted of 6 dogs (19 to 26 kg.) with ligation of the descending branch of the left coronary artery at various levels. The effect of nicotine on coronary blood flow was studied in these animals between the fourth and nineteenth days after ligation. These dogs showed in general very low control values for coronary blood flow (ranging from 30 to 50 cc./min.). During nicotine infusion there was only a slight and slow increase of flow between the third and sixth minute (up to 80 to 105 cc./min.). At the end of the infusion (15th minute) the coronary blood flow returned to the low control level and remained there until the termination of the experiment (for at least 15 min.).

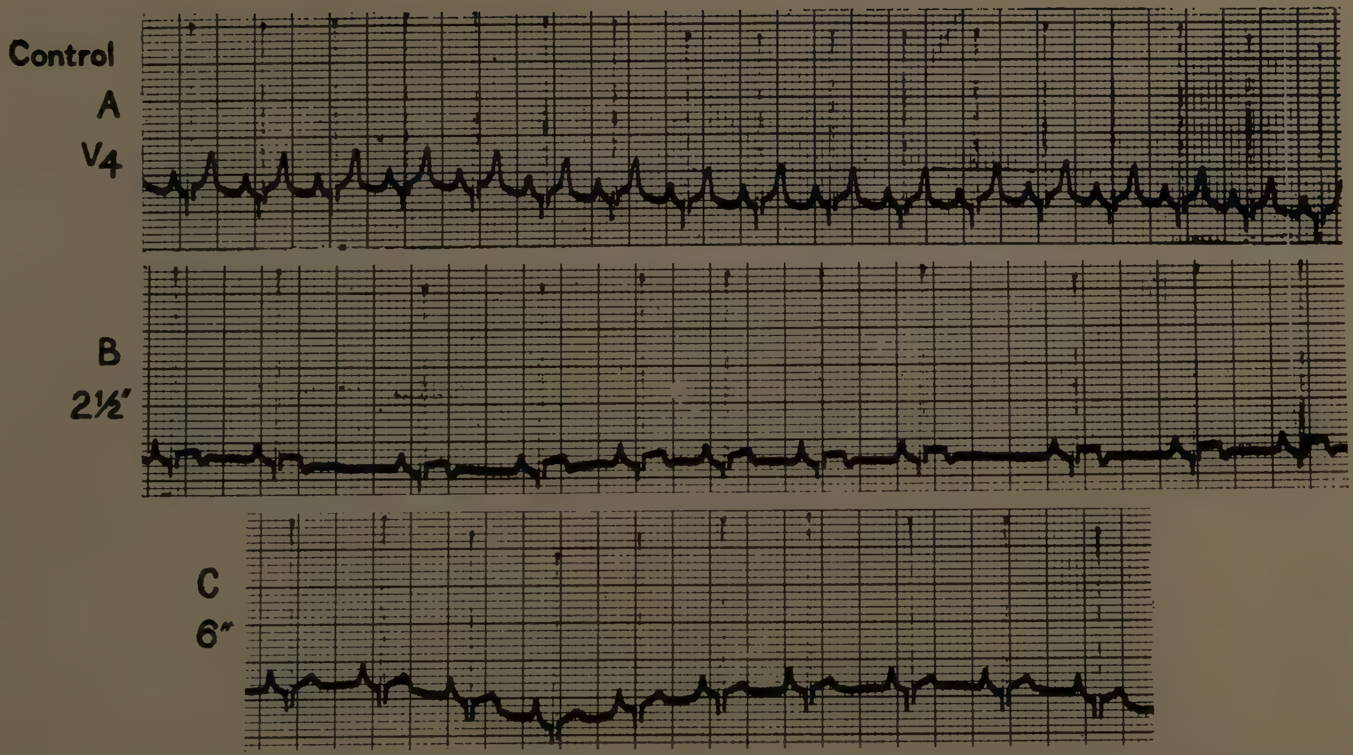

FIGURE 1. Effect of nicotine injection in a dog with coronary insufficiency. Casein rings were inserted February 25, 1960; the infusion of nicotine took place March 17, 1960. Control tracing V_4, shown at A, is within normal limits. The reaction of the experimental animal is shown at B, 2.5 min. after injection of 0.4 mg./kg. of nicotine intramuscularly. Note the elevation of the RT segment. C shows the same animal 5.5 min. after the injection. Note the upright T wave with no RT segment change. The T wave amplitude has not returned to normal.

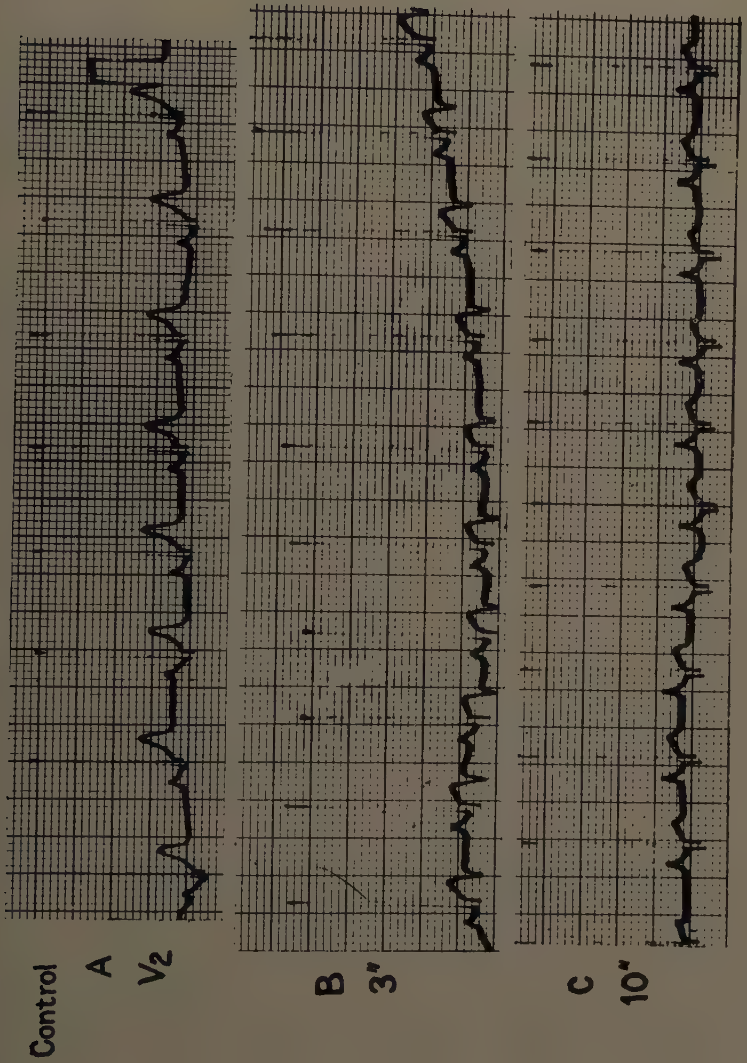

FIGURE 2. Effect of nicotine injection in a dog prepared as in FIGURE 1. Control tracing V_2, shown at A, is within normal limits. B indicates the reaction of the dog 3 min. after injection of 0.4 mg./kg. of nicotine intramuscularly. Note the elevation of the RT segment, with the terminal downward dip. In C, taken 10 min. after injection, note the return of the T wave to an upright configuration.

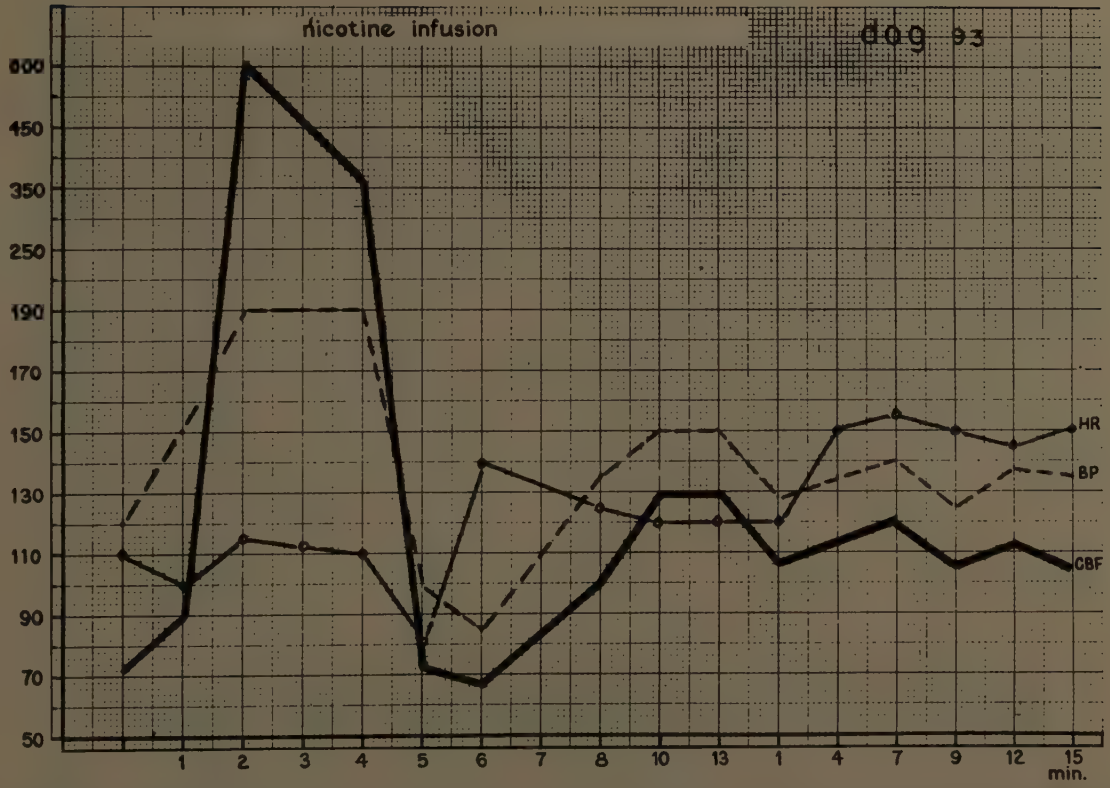

FIGURE 3. Effect of the injection of nicotine on coronary blood flow in the normal dog. Note the sudden increase in the coronary blood flow (CBF) between the first and fifth minute, from 70 to 600 cc./min. Following a dip down to the normal control, the CBF is increased above the normal control value, ranging between 110 and 130 cc./min. in the following 10 min. Note the increase in the heart rate (HR) and blood pressure (BP), which parallels the increase in the coronary blood flow.

The graphic expression of the blood flow showed a curve without a peak, but with a long "plateau." The response of the coronary blood flow appeared to be related to the acuteness and extent of the myocardial infarction: the more acute and extensive the myocardial infarction, the lower and broader was the plateau of coronary blood flow increase (FIGURE 4).

Cardiac output, cardiac work, and oxygen consumption of the myocardium increased; the coronary AV difference decreased.

Dogs with coronary insufficiency produced by casein rings. In these animals (8 dogs weighing 20 to 27 kg.) the control values of coronary blood flow tended to be lower than those of the normal dogs, but were in general higher than those of the dogs with coronary artery ligation. During the infusion of nicotine a slight and slow increase in coronary flow (10 to 30 cc./min.) was observed. This was significantly less than in the normal control animals.

The graphic expression of the coronary flow was that of an early low peak with a broad base. The early peaking resembled that of the normal dogs, but was much less marked. In addition, there was a tendency to form a plateau, as was observed in the dogs with coronary artery ligation (FIGURE 5).

Similar results were obtained with 8 dogs from whom coronary blood flow was obtained with the nitrous oxide method.[5]

Pathology. Dogs with ligation of the anterior descending branch of the left coronary artery showed anterolateral wall infarction in an acute or subacute stage, depending on the period of survival after the coronary artery ligation. The extent of the infarction varied from heart to heart, depending chiefly on the level at which ligation was performed.

The hearts wherein coronary insufficiency was produced by the casein ring inserted around both the circumflex and anterior descending branches of the left coronary artery showed the arterial lumen to be narrowed to a resultant diameter between 0.7 and 1.2 mm.; in some instances the lumen was almost completely obliterated. The pathologic examination of the heart revealed the following: (a) no gross areas of infarction; (b) areas of subendocardial necrosis with hemorrhages, confined especially to the lateral wall; or (c) areas of marked subendocardial fibrosis.

Discussion

The effect of nicotine and tobacco smoke on dogs with coronary insufficiency showed effects that were similar to those of a stress mechanism. The findings obtained in the electrocardiogram, namely RT segment elevations and the production of ectopic rhythm, are similar to those that are produced in the experimental animal and in the human subject resulting from emotional stress and/or exercise in the presence of coronary insufficiency.

Following the production of coronary insufficiency by coronary artery ligation and narrowing of the lumen of the coronary arteries, the pattern of response of the coronary blood flow was significantly different than that observed in the normal dog. The coronary blood flow showed only a slight increase as compared to the normal; this was also associated with a smaller change in cardiac output, coronary AV difference, and oxygen consumption. The response of the coronary blood flow was similar to that observed by Foltz *et al.*[6]

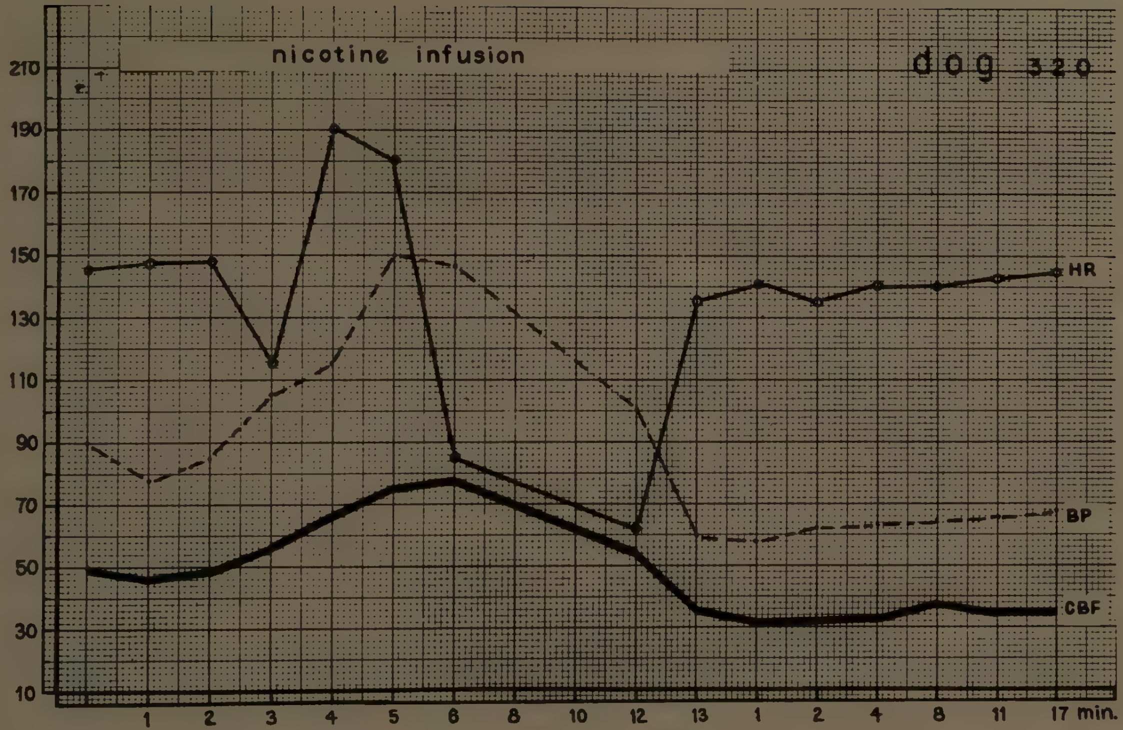

FIGURE 4. In a dog with coronary artery ligation, the initial low coronary blood flow (CBF) increases in a gradual manner following nicotine infusion, reaching a peak from the fifth to the eighth minute, and then gradually falling to a level below the normal control. Note the increases in the pulse rate (HR) and the blood pressure (BP) from the third to the twelfth minute.

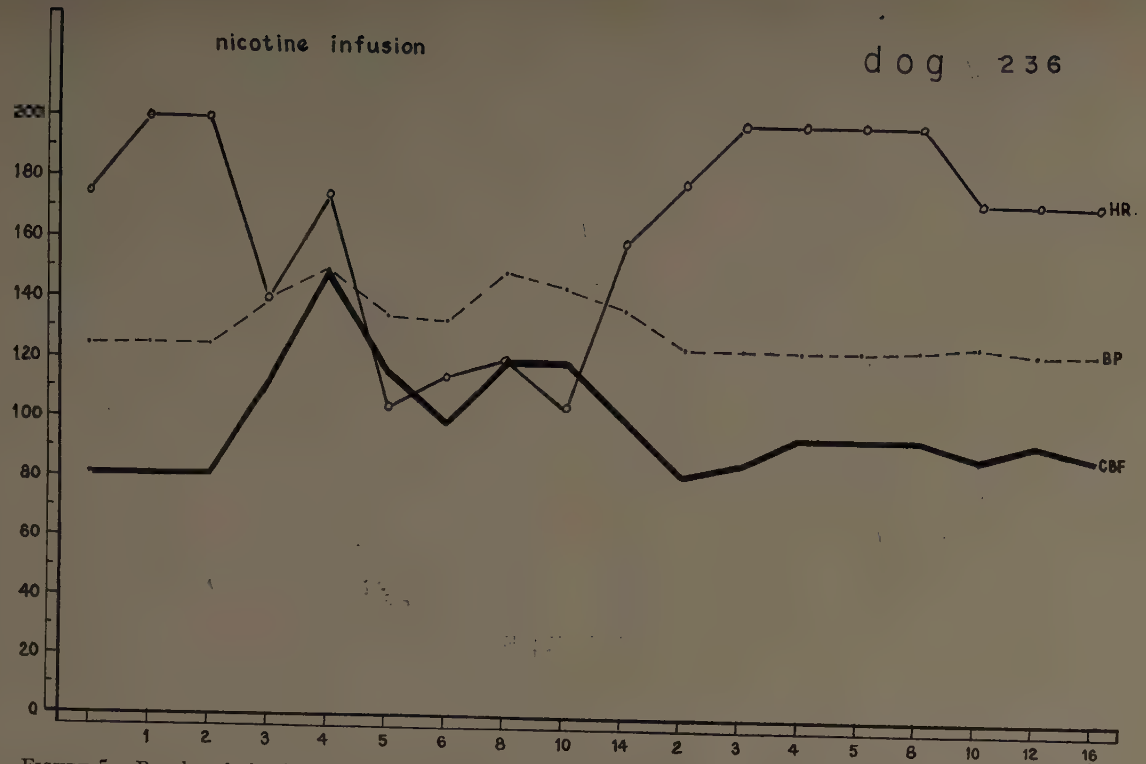

FIGURE 5. Results of nicotine infusion in a dog with coronary insufficiency produced by casein rings, one surrounding the anterior descending artery, and one in the circumflex branch of the left coronary artery. The relatively slight increase in coronary blood flow (CBF) reaches a peak at the fourth minute and gradually drops to the normal control level at the end of the infusion. Note the relatively high pulse rate (HR) and slight increase in blood pressure (BP).

relative to the effect of anoxemia in the presence of coronary insufficiency. These findings would appear to have a bearing on the clinical problem of the effect of nicotine and tobacco smoke in the presence of coronary insufficiency.

Summary

The effect of nicotine was studied in the normal dog and in dogs with coronary insufficiency as produced by coronary artery ligation and gradual coronary artery narrowing.

The response of the heart to similar infusions of nicotine showed a notable difference in the effect on the coronary blood flow and the measurement of other parameters including cardiac output, oxygen consumption, and coronary AV difference as compared to the normal control group. The increase in the coronary blood flow was considerably less than that in the normal animal and frequently dropped below the normal value in the postinfusion period. These findings would appear to have a bearing on the clinical problem relative to the effect of nicotine in the human subject with coronary artery disease.

References

1. Bargeron, L. M., Jr., D. Ehmke, F. Gonlubal, A. Castellanas, A. Siegel & R. J. Bing. 1957. Effect of cigarette smoking on coronary blood flow and myocardial metabolism. Circulation. **15:** 251.
2. Kien, G. A., N. Lasker & T. R. Sherrod. 1958. Action of cigarette smoke on cardiovascular hemodynamics and oxygen utilization in the dog. J. Pharmacol. Exptl. Therap. **124:** 35.
3. Stewart, G. N. & J. M. Rogoff. 1919. The action of drugs on the output of epinephrine from the adrenals; nicotine. J. Pharmacol. Exptl. Therap. **13:** 193.
4. Bellet, S., A. Kershbaum, R. H. Meade, Jr. & L. Schwartz. 1941. The effects of tobacco smoke and nicotine on the normal heart and in the presence of myocardial damage produced by coronary ligation. Am. J. Med. Sci. **201:** 40.
5. Bing, R. J., M. M. Hammond, J. C. Handelsman, S. R. Powers, F. C. Spencer, J. E. Eckenhoff, W. T. Goodale, J. H. Hafkenschiel & S. S. Kety. 1949. The measurement of coronary blood flow, oxygen consumption, and efficiency of the left ventricle in man. Am. Heart J. **38:** 1.
6. Foltz, E. L., W. F. Sheldon, R. G. Page, J. J. Sayen & W. T. Fitts. 1951. Quantitative circulatory response to an acute anoxemia test in dogs with progressive experimental coronary narrowing. Am. J. Med. Sci. **222:** 115.

TOBACCO ALLERGY IN CORONARY ARTERY DISEASE*

Joseph Harkavy and Ely Perlman
Mount Sinai Hospital, New York, N. Y.

Between 1933 and 1938 one of us presented evidence suggesting that tobacco hypersensitiveness could account for certain clinical syndromes involving the heart and peripheral blood vessels.[1,2,3] The conditions considered were thromboangiitis with migratory phlebitis, cerebral and coronary angiitis, sclerosis of peripheral blood vessels, and disorders of the heart, including extrasystoles, tachycardia, auricular fibrillation, "tobacco angina," and coronary artery disease with angina pectoris.[4] Prior to incriminating tobacco as a factor in these cardiovascular manifestations all other possible causes were excluded.

The idea that hypersensitivity to tobacco, tobacco smoke, and/or nicotine might be involved in the pathogenesis of these syndromes was prompted by the clinical observations that only certain individuals developed these symptoms from smoking, whereas others could smoke with impunity.

Allergy to nicotine has not been demonstrable to date. It was therefore felt that if hypersensitivity to smoking does exist, it would be due, apparently, to components of the tobacco leaf or their oxidation products. In the current study extracts of tobacco were prepared relatively free of nicotine and were used for immunological and clinical studies.

Preparation of Tobacco Extracts

Extracts were prepared of cured leaves of Burley, Maryland, Virginia, and Xanthis tobaccos. The leaves were shredded, defatted with ether, and extracted with Coca's alkaline extracting fluid. They were submitted to prolonged dialysis (10 to 14 days). This period was necessary to reduce the nicotine below detectable levels. The dialyzed extracts were lyophilized. Analysis revealed average values of the order of 7 per cent ash, 3.5 per cent nitrogen, and 40 per cent reducing sugars (after hydrolysis in $N/1$ HCl). No bound nicotine was detectable. Using a spectrophotometric method devised by one of us, the dried extracts were found to contain less than 5 μg. of nicotine per 100 mg. (0.005 per cent).†

Immunological Studies

In previous studies in rats[5] it was demonstrated that repeated injection of tobacco extracts was capable of sensitizing 8 of 11 rats, as indicated by a positive Schultz-Dale reaction and the development of digital necrosis in 5 of the group.[8] Nicotine did not produce any such reactions even after prolonged immunization. Female rats could not be sensitized.

In the present study, a total of 45 rabbits were immunized with each of the "nicotine-free" tobacco extracts. Some were given the tobacco extract intramuscularly in Freund's adjuvant, others were injected repeatedly using the

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* The work described in this article was supported in part by Research Grant 55 G 92 from the American Heart Association, New York, N. Y.

† Details of this method are to be published elsewhere.

intravenous route. Because the early studies proved the tobacco extracts to be poorly antigenic, 200 mg. were given in each of the later injections. Repeated bleedings were taken to ascertain the presence of antibodies. On various occasions one and two lines of precipitate were demonstrable using the Ouchterlony zone precipitation technique. There were some cross reactions among the four different types of tobacco leaf extracts. However, any one rabbit might have precipitins at a given time, only to lose them shortly thereafter.

Toward the end of these experiments 5 rabbits remained, each of which had had precipitins at one time or another. Three of these rabbits (101, 102, and 103), and two others (104 and 105) that were immunized but did not develop any demonstrable antibodies, and seven control rabbits were sacrificed for perfusion experiments. The isolated hearts of these animals were perfused according to the Langendorff[5] technique employing the Anderson-Craver apparatus,[6] with 10 to 20 mg. of tobacco extract in 2 ml. of perfusing solution and with 10 to 100 mg. of nicotine in the same volume.*

The results showed that the hearts of seven normal control rabbits perfused with Burley tobacco extract produced a variable, nonspecific effect on coronary flow (−79 to +112 per cent) and no significant effect on heart rate or contractile force. Of the three immunized rabbits (101, 102 and 103) with demonstrable antibodies, one (101) showed a significant decrease in contractile force (39 to 78 per cent), some decrease in heart rate (8 to 18 per cent), and a consistent decrease in coronary flow (65 per cent) when perfused with Burley tobacco extract. Maryland and Virginia tobacco extracts produced no significant difference in this rabbit, suggesting the possibility of immunization rather than sensitization to these tobaccos.

The heart of rabbit 103, although negative on perfusion, showed a grayish apical lesion on gross examination. Microscopic section revealed an area near the apex consisting of bundles of cardiac muscle fibers undergoing a variety of degenerative changes, including necrosis and replacement by cellular fibrous tissue. Slightly hyaline medial changes were noted in an occasional small myocardial artery, and one arteriole showed a uniform smudgy alteration throughout the wall.

The isolated hearts of rabbits 104 and 105, previously injected with Burley, Maryland, and Virginia tobaccos, against which they had not developed any demonstrable antibodies, showed no cardiac changes on perfusion with the homologous tobacco extracts. However, the hearts of these two rabbits were found to be refractory to nicotine, in that doses as high as 200 μg. caused no reactions.

Challenge of the excised ileums of these rabbits with the tobacco extracts in question yielded negative Schultz-Dale reactions. This may be explained by the inability of the smooth muscle of the rabbit's intestine to fix antibody. Consequently it is indifferent to direct antigenic impact.[6]

It would seem from these experiments on rats and rabbits, and from the demonstration of precipitins in rabbit sera, that tobacco leaf extract is antigenic, although poorly so, in these animals.

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* The perfusion experiments were carried out by M. Finkelstein of the Pfizer Laboratories Division of Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

Clinical Studies

During the course of the previous and current studies of patients with cardiovascular syndromes suspected of being due to tobacco hypersensitivity, the following evidence has been accumulated which seems to satisfy the criteria for establishing the existence of an allergic etiology:

(1) It has been shown clinically that in certain individuals smoking was consistently followed by the development and exacerbation of their symptoms. Certainly in thromboangiitis obliterans, the smoking of one cigarette can be followed by phlebitis. One patient developed a recurrence of previous phlebitic nodules 24 hours following skin tests with tobacco extracts, while another developed a recurrence of thromboangiitis and a generalized eczema following repeated injections with tobacco extract.[7] In cases with cardiac arrhythmias, tobacco angina as such, and coronary artery disease with anginal pain following smoking, persistence in the use of tobacco led to continuation of symptoms as well as progression of the coronary artery disease. Some of the patients with coronary disease who did not stop smoking had three and even four attacks of coronary artery disease, which brought them repeatedly to the hospital.

(2) It has also been demonstrated that removal of the allergen (that is, tobacco) results in a permanent arrest of symptoms.[4] This has been shown to be true in patients with cardiac irregularities of rhythm, such as extrasystoles, tachycardia, and auricular fibrillation, and also in patients with thromboangiitis obliterans. In individuals with angina, pain due to smoking also disappeared following the elimination of tobacco.

(3) Positive skin tests to tobacco are demonstrable in a high percentage of patients with cardiovascular symptoms suspected of being allergic to tobacco.[4]

(4) The existence of reagins to tobacco in sensitive patients has been confirmed by positive passive transfer reactions, as well as by specific absorption of reagins by different tobaccos and the presence of eosinophilia in the immediate whealing reaction.[9,10]

In our present investigation we tested with the above described specially prepared "nicotine-free" tobacco extracts a group of 57 patients admitted to the hospital because of coronary artery disease and myocardial infarction. These patients were chosen sequentially from among all such patients admitted to the wards. Each individual was tested with ragweed pollen, Burley, Maryland, Virginia, and Xanthis tobacco extracts. The results of these and of similar studies in 49 cases of coronary artery disease carried out in 1935, utilizing 70 per cent denicotinized tobacco, are indicated in TABLE 1.

It is evident from this table that 34 (59.6 per cent) of the 57 cases in the 1959 group who smoked gave positive skin reactions suggesting allergy to one or more tobaccos. Seventeen smokers (29.6 per cent) showed negative reactions, as did all of the 6 (10.75 per cent) nonsmokers.

The average age at onset of acute coronary artery involvement in smokers who showed positive skin tests to tobacco was 56+, whereas in smokers not sensitive to tobacco it was 65 years, a difference of 9+ years. The average age at onset of acute coronary artery diseases in nonsmokers with negative skin reactions to tobacco was 69+, a difference of 13 years between this group and the tobacco-reacting smokers.

Comparison of the findings in 49 coronary artery cases reported in 1935[4] (tested with 70 per cent denicotinized tobacco extracts) with those in 1959, revealed that the age at onset of acute coronary symptoms in 22 smokers with positive skin reactions was 49+ years, whereas in 18 smokers not reactive to tobacco it was 59.8, a difference of 10 years. In 9 nonsmokers the initial coronary symptoms appeared at the average age of 68.7, a difference of 19 years compared to smokers with coronary artery disease who reacted to tobacco.

The results noted in both categories of patients comprising this group were submitted to statistical analysis and, by using the *t* test, showed a P of 0.01, which is highly significant.

It is interesting to note that there seems to be an age differential in the onset of coronary artery disease in the 1935 group compared with the 1959 group. Thus in the interval of 24 years, the age at onset in the tobacco sensitive group shifted from 49+ in 1935 to 56+ in 1959 and, in the nonsensitive smoker, from 59.83 in 1935 to 65+ in 1959. There was but little change in coronary involvement in the nonsmoker group: for example, 68.7 in 1935 and 69+ in 1959. The shift to the later age in onset of acute coronary involvement in the tobacco sensitive and nonsensitive groups in the interval of 24 years may be explained on the following grounds: (1) the life span of the population has generally increased in the past quarter of a century; and (2) even though coronary artery disease in smokers appears more frequently in younger individuals[11] it is possible that this group of patients, because of hospitalization insurance (not available to, or not acquired by older individuals) do not appear in public wards, but obtain private care.

TABLE 1

	1959: 57 cases		1935: 49 cases	
	No. of cases	Avg. age at onset	No. of cases	Avg. age at onset
Smokers				
tobacco-sensitive	34 (59.6%)	56+	22 (44.7%)	49+
tobacco-insensitive	17 (29.6%)	65+	18 (37.0%)	59+
Nonsmokers	6 (10.8%)	69+	9 (18.4%)	68+

Discussion

In view of the observations described above it would seem that exposure to tobacco, whether through smoking or by injection, may be followed by sensitization in the experimental animal as well as in man. In animals it may be demonstrable by anaphylactic reaction in various tissues, such as smooth muscle, the heart, and blood vessels. In man hypersensitivity to tobacco is indicated by the presence of positive skin reactions to various tobaccos.

Clinically, tobacco allergy may be responsible for symptoms in various shock tissues that are peculiar to the affected individual. In some the respiratory system may react, giving rise to cough and or asthma; in others the blood vessels in the brain, resulting in migraine; and in still others the cardiovascular system may be implicated. Evidence of the influence of allergy, in the predisposition to coronary artery involvement in the tobacco sensitive group of patients, is to be found not only in the positive skin reactions to tobacco and passive transfer experiments with sera of patients with tobacco angina, coronary insufficiency, and coronary artery disease, but also in the histories of these patients. Investigation disclosed that 33 to 34 per cent of the 1959 and 1935 groups of patients with cardiac manifestations had a family or personal background of allergy. This relatively high incidence of constitutional stigmata of allergy in cases with coronary artery disease deserves due consideration. It implies that in such allergic individuals the coronary vessels and the myocardium constitute the shock organ, and that abnormal reactions in these tissues may be the results of hypersensitiveness to various exciting agents, one of which, and in certain cases the most important, may be tobacco. This is also supported by the history of precordial pain after smoking in 32 per cent of the 34 cases in 1958–1959 group that gave positive skin tests to tobacco. Unfortunately, information on this point was not recorded in the 1935 series of patients. While such a positive history is important in correlating the symptoms and positive skin tests, its absence does not exclude tobacco hypersensitiveness. Fontana *et al.*[12] have recently reported that 33 per cent of 96 smokers with a personal history of allergy and positive skin reactions to tobacco developed peripheral vascular symptoms, as compared with 10 per cent of 291 smokers with negative skin reactions to tobacco. In other words, there was a consistent parallelism between positive skin reactions to tobacco extracts and allergic manifestations, such as coughing spells, as well as numbness and tingling of the extremities as a result of smoking, suggestive of an allergic mechanism. These observations confirm our previous reports along those lines. However, it should be noted that some individuals, although sensitive to tobacco as indicated by clinical manifestations following smoking, may give negative skin reactions to this allergen. This is a common experience when dealing not only with tobacco allergens but with any other allergens used in testing hypersensitive patients. Under such circumstances the diagnosis of specific hypersensitiveness is made by the exclusion of a suspected allergenic excitant and the effects noted following its elimination.

Summary

Evidence has been presented that denicotinized tobacco is anaphylactogenic in experimental animals such as rats and rabbits. In rats this is demonstrable by reactions in smooth muscles (Schultz-Dale reaction) and peripheral blood vessels (digital necrosis). In rabbits it is evident in the presence of precipitins to tobacco, in the reduction in coronary flow as well as contractile force of the heart and, to a lesser extent, in the heart rate on perfusion (rabbit 101); and in the finding of an apical necrosis (rabbit 103) that is in keeping with similar lesions noted in sensitized cardiac tissue.

Tobacco hypersensitivity as a cause of cardiovascular disorders in man is supported by the following observations: (1) exposure to tobacco provokes symptoms in the specific shock organ, that is, the heart and blood vessels; (2) abstention from smoking causes clinical remissions; (3) positive skin tests are

correlated with suspected clinical allergy; (4) reagins to tobacco have been demonstrated by passive transfer tests; and (5) the reagins are specific for various types of tobacco.

Investigation of 57 patients with coronary artery disease during 1958–1959 indicated that the onset of acute coronary involvement in 34 smokers (59.6 per cent) with positive skin reactions to tobacco occurred nine years earlier than in 17 smokers (29.6 per cent) who showed negative reactions to tobacco, and 13 years sooner than in nonsmokers with negative skin tests. Similar results were obtained in 49 cases studied during 1935.

Statistical analyses of these findings were found to be highly significant.

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References

1. Harkavy, J. 1933. Bull. N. Y. Acad. Med. **9**(5).
2. Harkavy, J. 1934. J. Allergy. **5**: 131.
3. Harkavy, J. 1938. J. Allergy. **9**(5): 475.
4. Harkavy, J. 1946. Clinics. **5**(2): 504.
5. Langendorff, O. 1895. Arch. ges. Physiol. **61**: 291.
6. Anderson, F. F. & B. N. Craver. 1948. J. Pharmacol. Exptl. Therap. **93**: 135.
7. Harkavy, J. 1938. J. Allergy. **9**(3): 275.
8. Lecomte, J. & J. Hugues. 1954. Rev. Med. Liege. **9**: 276.
9. Harkavy, J. & E. Witebsky. 1935. J. Allergy. **6**: 437.
10. Harkavy, J. 1938. J. Allergy. **9**(5): 475.
11. North Dakota Study on the Epidemiology of Coronary Heart Disease. 1959. Am. J. Public Health. **49**(12): 1630.
12. Fontana, V. J., W. Redisch, R. L. Nemir, M. K. Smith, K. DeCrinis & M. B. Sulzberger. 1959. J. Allergy. **30**: 241–249.

Part VIII. Panel Discussion

EFFECTS OF SMOKING IN DISEASES OF THE PERIPHERAL VASCULAR SYSTEM

E. A. Hines, Jr., *Moderator*
Mayo Clinic, Rochester, Minn.

E. A. Hines, Jr.: Although our title is "Effects of Smoking in Diseases of the Peripheral Vascular System," we shall be concerned with effects of tobacco however it may be introduced into the human. Peripheral vascular diseases in our discussion will include diseases of the lymphatic system (vascular diseases that we often overlook) and also hypertensive vascular disease.

We shall first consider the ways in which tobacco may be helpful or harmful in peripheral vascular diseases. We shall try to apply some of the basic physiological and biochemical information, which has been so well given at this meeting, to the physicians' problem in caring for his patient with peripheral vascular disease. In those conditions where we agree that tobacco may have a harmful effect, we shall suggest what can be done about it, either in the nature of a deterrent to the effect of tobacco on the peripheral vessels, or in the nature of a deterrent to the patients' tobacco habits.

To review briefly the effects of tobacco that might (and I emphasize might) be beneficial, I mention the tranquilizing effects that are mentioned several times in this monograph; the antiobesity effect; the possible cholesterol-lowering effects of nicotine; and, perhaps, the increase of blood pressure in conditions such as orthostatic hypotension where pressure is low. On the other hand, some of the ways by which tobacco might have a harmful effect in peripheral vascular diseases include inducing or increasing vasoconstriction; increasing the tendency to thrombosis; inducing or increasing the changes of atherosclerosis by its effect on fat metabolism; and an allergic reaction.

I ask Edwin Wood if all the effects of tobacco in peripheral vascular diseases can be explained by the action of nicotine?

Edwin Wood (*Medical College of Georgia, Augusta, Ga.*): Yes, I think they can. This is not to say that all of the other substances in tobacco have no effect on the peripheral circulation, but evidence appears in this monograph indicating that nicotine can reproduce the observable effects on the circulation, or that knowledge of the pharmacology of nicotine can explain changes that we observe in the circulation.

Hines: Do any of the other members of the panel have any comments on this?

Hugh Montgomery (*University of Pennsylvania, Philadelphia, Pa.*): I think it is still an open question whether, as Harkavy has mentioned, some effect on collagen might not play a part in some peripheral vascular diseases. I think thromboangiitis obliterans has not been disproved as being a result of some sort of allergy, and its very close association with the use of tobacco, although not 100 per cent, is still suggestive.

HINES: In the main, however, I think we can concentrate our discussion on the effects of nicotine.

QUESTION: If we can get down to some more exact information, what is the least amount of nicotine that might cause adverse effects on the blood vessels?

GRACE M. ROTH (*Lovelace Clinic, Albuquerque, N. M.*): The standard cigarette contains 20 mg. of nicotine and about 22 per cent of that—or about 4.4 mg.—is in the mainstream of the smoke that enters the mouth. In doing the studies with the low nicotine content cigarette, we found that when the amount in the mainstream was 0.55 mg. we had only a few vascular effects. That probably means that the denicotinized cigarette must be cut down about 60 per cent to avoid or banish the vascular effects, and that is why the denicotinized cigarettes have given almost the same effect as the standard cigarette.

HINES: Does tobacco have a tranquilizing effect in the hypertensive patient that might tend to cancel some of the beneficial effects of stopping the use of tobacco?

ROBERT H. GOETZ (*Albert Einstein College of Medicine, New York, N. Y.*): It depends on what you mean by tranquilizing effect. If it is considered essential that a tranquilizer calm down a patient's functions, such as the heart rate, or keep the blood pressure at a constant level, then I think nicotine or smoking cannot be regarded as being a tranquilizer. After all, it does increase the heart rate and the blood pressure. I noticed in Roth's discussion in this monograph that the diastolic pressure went up as much as 70 mm.

On the other hand, if a patient is confronted by something that irritates him, or is apprehensive, he is likely to start smoking. Apprehension and irritation increase the blood pressure and heart rate and that seems to be a poor time to add the vasoconstrictor effects induced by smoking. Therefore, although smoking may perhaps make the patient feel better mentally, from a physiological standpoint tobacco is not a tranquilizer and should not be recommended for that purpose. A different type of tranquilizer should be used if one is needed.

HINES: Does anyone on the panel know of evidence showing that when a patient with hypertension has stopped smoking his blood pressure goes up from the tension and anxiety of stopping the use of tobacco? I don't know of any such evidence, and I doubt that this would occur.

ROTH: An interesting piece of work was done by Johnston[5] in England. Johnston, with his 35 colleagues, who were habitual smokers, observed the effect of substituting nicotine. When they wanted a cigarette, they took a 0.02 mg. of nicotine hypodermically. After about 35 injections, they preferred to have their nicotine by way of the hypodermic needle rather than by smoking. They decided from this that the tranquilizing effect they experienced from smoking was secondary to the satisfaction of the desire for nicotine that had been lacking. This conclusion was based on a small group of subjects, but is the only piece of work of this kind of which I know.

HINES: Can Hugh Montgomery tell us whether the weight gain after stopping smoking tend to cancel some of the beneficial effects of stopping the use of tobacco?

MONTGOMERY: It was interesting that, in this monograph, Blackburn reported an average 9-lb. weight gain when a person stops smoking. That has been just about my impression. It is a difficult problem when a middle-aged businessman, with a condition such as intermittent claudication from a blocked artery, comes for advice. Such a patient may weigh a little more than he should. He has been smoking a couple of packs of cigarettes a day. Which is more important—to get his weight down, and perhaps decrease his cholesterol in so doing, or to stop the smoking? It is usually very difficult for him to do both. Maybe I should be a nonsmoker and I then should be more effective, but practically speaking, I think I fail more often than I succeed in having a person do both at the same time.

HINES: I'll agree it is very difficult to do both at the same time. Such gain in weight, according to statistical studies, is not large enough to be a greatly increased health hazard. Nine pounds would not be beyond the 10 per cent that supposedly results in an increased health hazard.

MONTGOMERY: However, suppose the patient weighs 250 pounds and this represents the major problem. Then would you not let him smoke and try to get his weight down?

HINES: No, I would try to persuade him to do both.

MONTGOMERY: I don't remember any recent occasion in which I was successful in scoring on both points.

HINES: May we say then that the panel agrees that the beneficial effects in peripheral vascular disease of tobacco are minimal and somewhat nebulous, and should never overweigh the stopping of tobacco in conditions where there is a definite contraindication? In other words, would stopping the use of tobacco take precedence over anything else? Are there any really beneficial effects that might be derived from continuing the use of tobacco? It seems from the lack of response that we are in agreement on that.

WILLIAM WOLF (*Grand Central Hospital, New York, N. Y.*): I was wondering whether any one on the panel could tell us the mechanism of the fat accumulation that occurs when one stops smoking and, contrarywise, what the loss of fat is due to when one does smoke?

HINES: I shall have a little trouble answering that because I am a nonsmoker. I can say, though, that I have trouble keeping my weight down, and I think one reason is because instead of lighting up a cigarette, I eat a piece of candy or something of that nature. So I think just substituting the eating habit for the smoking habit is one reason why patients who stop smoking gain weight.

Smokers will have to say more about the effect on appetite and other things that might be involved.

WOLF: Thienes pointed out in his paper that the rats he studied ate the same amount in each instance. So we must consider absorption and other factors as well.

MONTGOMERY: We are not rats, and I am told that nicotine suppresses hunger contractions as does smoking. Is that an incorrect statement?

HINES: I think that is correct.

MONTGOMERY: Nicotine releases a little of the body sugar instead of letting the sugar go in by way of the mouth.

HINES: I think there is an effect on the appetite from smoking.

GOETZ: That is the way I have always explained it, because the first thing a smoker does when he feels hungry is to reach for his pack and start lighting a cigarette, so that instead of having his lunch, he smokes. I am a nonsmoker and usually eat something in order to get over my hypoglycemia at about half-past eleven in the morning, whereas my colleagues always smoke at that time. Eating is, I think, the oldest tranquilizer, in fact one of the best tranquilizers known.

WOLF: What happens to the blood pressure if a person has been a habitual smoker and then stops smoking for, perhaps, a year or six months, and then starts again? Are these changes in any way comparable?

HINES: Does anybody on the panel have an answer to that one? I don't recall any studies on it.

MONTGOMERY: May I make one remark apropos of that. Two of my patients who had previously been heavy smokers, drove their cars off the road during the first 2 or 3 days after stopping smoking. Apparently, they just collapsed. I do not know whether it was a blood pressure effect or a slowing of the pulse or something purely psychic. One of them did it twice after stopping smoking. Luckily, neither of them was killed, but the accidents seemed to be connected with the abrupt withdrawal.

BÖRJE EJRUP (*Karolinska Sjukhuset, Stockholm, Sweden*): We have seen in Stockholm about 2500 patients who have stopped smoking, and we have not seen any trouble like that.

HINES: I don't know of any patients who have had that experience. It must be a pretty rare occurrence. May I ask Hugh Montgomery if there is any evidence that nicotine can accelerate clotting of the blood inside the blood vessels.

MONTGOMERY: I was interested in hearing Wenzel say that there is a decrease in the clotting time, *in vitro*, not in the blood vessels, and again in rabbits and not in man.

Orville Horwitz in our laboratory is working on clotting mechanisms apropos of the use of cigarettes, and his impression at the moment is that there is one test in which he has been able to show in a number of patients a distinct tendency toward increase in clotting rate during the smoking of a single cigarette.

GOETZ: I cannot quote chapter and verse, but I believe that somebody many years ago found that the viscosity of the blood was changed on smoking.

HINES: We can say that the evidence is not good enough at the present time to permit us to use this as a reason for urging a patient, who has a recurring thrombosing tendency, to stop the use of tobacco.

Since I made a point that lymphatics are vessels and that there is such a thing as lymphatic disease, I ask Wood if there is any evidence that tobacco is helpful or harmful for patients with lymphatic vascular disease.

WOOD: Victor McKusick, John Esterly, and I[1] had an opportunity to study patients with Milroy's disease (chronic hereditary lymphedema). These patients have very high rates of blood flow that conceivably could contribute to the edema formation. Possibly smoking would alter this situation, but this is purely speculative.

HINES: There is a great deal of further study needed on this effect. Certainly lymph vessels can contract and dilate like blood vessels and it is possible that nicotine might have some effect on their function.

As regards one of the major situations in regard to tobacco and peripheral vascular disease, what evidence is there that tobacco is harmful in patients with thromboangiitis obliterans or Buerger's disease?

MONTGOMERY: The best study I know of is that made about 20 or 30 years ago by Silbert,[2] whose results pointed very clearly to the importance of giving up the use of tobacco in all forms. The group consisted of about 200 patients with thromboangiitis obliterans, some of whom he followed for as long as 10 or 20 years; he found that about half of them gave up all use of tobacco while the other group continued to smoke, at least to some degree. In all of those who stopped smoking there was no advance of the arterial occlusions, while all who continued to smoke had very easily recognizable increments in occlusions and in ulceration.

There are exceptions. I have one patient who was under observation for five years while he smoked and there was no measurable advance in his disease, and another who, there is every reaon to believe, never smoked. They are the exceptions rather than the rule.

HINES: I do not particularly like testimonials as a substitute for statistics, but I mention several informative cases. Gifford and I reported[3] the case of a patient with thromboangiitis obliterans who had recurring episodes of superficial phlebitis and progression of occlusive arterial disease over a period of about 3 or 4 years, during which time he continued to smoke. We did not see him again for 10 years; during this period he had stopped smoking. The patient had had no further recurrence of his phlebitis. He had no evidence of occlusive arterial disease. It is impossible to be certain, but it certainly looked like a "cure."

We also have in our records the case of a smoker who, when he was 18 years old, had recurring superficial phlebitis, occlusive arterial disease, and gangrene of a toe that required amputation. This patient stopped smoking and did not resume smoking until he was 37 years old. Within six months he had a recurrence of superficial phlebitis and gangrene of another toe that required amputation. I do not blame him for thinking he was cured after that period of time.

We have another patient who had stopped smoking for 13 years and, on starting again, had recurrent symptoms and signs of active thromboangiitis obliterans.

GOETZ: I can only underline what has already been said. However, we all have seen cases of what appear to be thromboangiitis obliterans, that is, young males with no signs of arteriosclerosis or atherosclerosis and no increase in lipids and cholesterols who exhibit typical migrating phlebitis and subsequently develop thromboangiitis obliterans although they never smoked. A patient of mine, a young Irishman with migrating phlebitis, had never smoked in his life. We did an arteriogram and demonstrated the development of an intraluminal thrombosis. The vessel was still patent, but we could see the typical segmental clot in the posterior tibial artery in the middle of the vessel. Two weeks later he developed complete occlusion of that vessel and a biopsy showed the typical picture of thromboangiitis obliterans.

To make another point, it is noteworthy that patients in whom we make a diagnosis of thromboangiitis obliterans find it extremely difficult to stop smoking. Such patients will sit in bed with their legs flexed, suffering severe pain, and will smoke one cigarette after another, although they have been warned that they will lose their legs unless they give up smoking. They just are unable to stop.

HINES: There are many patients with thromboangiitis obliterans who do stop smoking and have not smoked over a period of many years. Roth and I know of a number of them.

ROTH: There have been some warnings put out about the filter-tip cigarettes, in that some of these people who formerly had Buerger's disease have gone back to smoking, thinking they were protected.

HINES: I think that is an important point. We should always tell our patients that filter-tip cigarettes do not take out enough of the nicotine to allow them to continue to smoke without harmful effects.

Next, what evidence is there that tobacco is harmful for patients with arteriosclerosis obliterans? Until recently it has been a clinical impression and, I think, a well-established clinical impression that, as a group, patients with arteriosclerosis obliterans who stopped smoking did better in that there were fewer complications, and fewer patients lost an extremity than in the group who continued to smoke. Joergens, Barker, and I,[4] and our associates, have been following patients with arteriosclerosis obliterans. Incidental to other information on two of these studies, we obtained information on the smoking habits of these patients over a period of years for an average of 13 years after they were first seen at the clinic. In one group in which the diabetic patients were excluded, which makes some difference, there were about 56 patients who had stopped smoking and about an equal number who had continued to smoke. Of those who had stopped smoking, not one had lost an extremity and, of those who had continued to smoke, 12 per cent had lost an extremity.

In another study (submitted but not yet published) that Schadt and I have done on patients with atherosclerotic occlusion of the femoral artery, an unselected group that included patients with diabetes, there were about 90 who had stopped smoking, and one of these had lost an extremity. There were about 85 who had continued to smoke, and 20 per cent of these lost an extremity. This cannot be considered as evidence that tobacco and smoking alone caused them to lose an extremity, but it gives some objective support for the general impression that patients with arteriosclerosis obliterans who continue to smoke do not do as well as those who stop smoking.

WOOD: Did sympathectomy "protect" these patients, as acute experiments would suggest; were there enough with sympathectomized limbs so that you could get this information?

HINES: No, there were only about 20 in this group who had sympathectomies.

MONTGOMERY: According to the recent paper by Joergens, Barker, and yourself,[4] about three quarters of the patients with peripheral arterial disease, who were followed through to death, died of coronary occlusion. Is it true that these deaths occurred earlier in that group?

HINES: Yes, the deaths were earlier on an average in smokers than in the nonsmokers.

We should like to have more objective clinical evidence that tobacco is harmful for patients with arteriosclerosis obliterans. I ask Wood to comment on the problem of evaluating the question of a causal relationship between tobacco and atherosclerosis.

Wood: The problem is that there appears to be a greater incidence of death from coronary disease in smokers than in nonsmokers. It seems to me that of the important problems in such evaluations, one is that people who elect to smoke do so on a psychological basis. I wonder if factors that determine whether or not a person will have an accelerated course of arteriosclerosis are also factors that independently determine whether or not he will be a smoker.

Another problem is one of evaluation of the disease. We have attempted to get better information by trying to develop a method for measuring arterial elasticity and obtaining a quantitative evaluation of arteriosclerosis, that is, by studying its course rather than by using a single coronary occlusion as a criterion.

These are some allusions to the problems rather than solutions.

Hines: It is well to emphasize that there are problems in trying to evaluate a possible causal relationship of atherosclerosis and the use of tobacco. It is going to be quite a long time before we can hope to have specific answers because of the circumstances mentioned.

To discuss other conditions in which tobacco might be a factor: when should a patient who is a marked vascular hyperreactor or who has hypertensive disease be advised to stop the use of tobacco?

Roth: That is difficult because people who are smoking want to continue to smoke! One of the best ways I have found is to do a smoking test on a patient and show him how his blood pressure rises every time he smokes a cigarette. Generally it rises about the same amount as in the cold pressor test, since anyone who gives a hyperreaction to smoking has a hyperreactivity to almost everything. A man whom I saw about 10 years ago had a blood pressure of 110/70 that went to 170/110 on smoking. He continued to smoke and the disease continued to progress. There are many such patients. The best that we can do is advise them to stop smoking and emphasize the fact that the patient's heart and blood vessels are at stake.

Hines: In a patient who is a vascular hyperreactor, I go only so far as to advise that, if he does not smoke, he should not take up smoking. I do not think we have nearly enough evidence of long-term harmful effect in a vascular hyperreactor from smoking to tell a hyperreactor that he should stop smoking.

Montgomery: Does the word hyperreactor, as we are using it in this discussion mean hyperreactive blood pressure?

Hines: Yes.

Montgomery: Is that not a misuse of the term? There certainly are a lot of different hyperreactions that can occur. Occasionally in smoking, for instance, a person will get a cold, sweaty reaction.

Hines: We are now concerned specifically with blood pressure hyperreactors.

Roth: I was speaking of the patient whose blood pressure is not usually elevated but goes up and down. There are many patients who can have a rise of 100 mm. in their blood pressure.

Hines: As with any strong stimulus, whether it is cold, pain, nicotine, or something else, there is an occasional patient whose blood pressure is very hypersensitive. In the case of tobacco, such a patient will have a persistent elevation while smoking and, when not smoking, will have normal blood pressure. This is a rare occurrence, but it should be sought. If there is any question about whether a patient who has hypertension or who is a marked blood pressure hyperreactor should stop smoking, there is a way to get some objective information: that is to conduct a smoking test under controlled circumstances and find out whether tobacco is keeping the blood pressure elevated.

Is there evidence that vasoconstriction induced by nicotine is harmful for patients with vein diseases, such as thrombophlebitis or primary varicose veins, or whether this mechanism is involved in congestive heart failure?

WOOD: I shall confine my remarks to constriction of the veins, based on the work of Eckstein and Horsley presented elsewhere in this monograph. We thought we had good evidence in Robert W. Wilkin's laboratory in Boston, Mass., that increasing the velocity of flow through the veins with elastic stockings actually lowered the incidence of thrombophlebitis. Thus if one accepts the idea that blood flow in the calf is not influenced by smoking, and that the veins constrict, then velocity would increase, suggesting at least that, if anything, it would be better to smoke. It would be unreasonable to think that the vasomotor effects of smoking induced thrombophlebitis. The effects on the clotting mechanism, if any, do not appear to be well studied.

With regard to congestive heart failure, blood volume is elevated and we have found evidence that the veins are constricted; hence venous pressure is high. We have all seen patients with congestive heart failure who say they get even shorter of breath when they smoke. This has generally been attributed to the irritant effects of tobacco on the bronchi. I suggest that, on the basis of the finding that blood is shifted out of the extremities by nicotine, this might contribute to the symptoms observed in congestive heart failure.

HINES: The most common symptom that patients with occlusive arterial disease complain of is intermittent claudication. What is the effect of nicotine on intermittent claudication?

MONTGOMERY: I do not think anybody has demonstrated that in man smoking affects intermittent claudication one way or another. One might put it around the other way and say that since we have shown that resting muscle blood flow is increased in a transitory manner by intravenous nicotine, it might well be that drugs such as Arlidin, for instance, which has been shown to increase resting muscle blood flow, should benefit intermittent claudication. I do not think that any drug increases the walking distance in intermittent claudication. I am reasonably sure that nicotine does not. I think that the controls of reported experiments that point to the contrary are not good, and I think there is no greater stimulus to an increased blood flow in muscle than the accumulation of metabolites that occurs in intermittent claudication.

GOETZ: May I confirm what Montgomery has said. I have the feeling that the increase in blood flow in muscle while smoking is a passive effect. Furthermore, the increase is really minimal when compared with the increase produced by the metabolites during exercise. There is no evidence that the flow of blood in the exercising muscle is increased by smoking and, consequently, it is unlikely that smoking has any effect on the exercise tolerance in intermittent claudication.

HINES: From a clinical standpoint, we do not have any well-controlled statistical studies on what happens to intermittent claudication when a patient stops smoking. Some patients may be able to walk farther when they stop smoking but, in most, it does not help. I think it is very important to point out to the patient when he is told to stop smoking that he probably will not be able to walk much farther; from this he should not assume that he is not being benefited and resume smoking. It should be made clear to the patient beforehand that the purpose of stopping the use of tobacco is not to increase his walking capacity.

Should all patients with ischemia of an extremity severe enough to endanger the survival of the extremity be urged to stop the use of tobacco?

GOETZ: Emphatically, yes. It is quite obvious that by the time the patient has ischemic lesions, anything that will further decrease the peripheral blood flow definitely will be harmful and, for that reason, I think it is absolutely essential for the patient to stop smoking. He should, of course, avoid anything else that will produce vasoconstriction, such as cold.

HINES: Does anyone disagree with this opinion?

I should like to stop now to summarize briefly and see if the panel agrees with me on which patients with peripheral vascular disease should be advised to stop the use of tobacco. Patients who are hyperreactors in their blood pressure or who have hypertension, in which it can be proved by testing that the use of tobacco is causing a persistent elevation of blood pressure, are one group that could be advised not to smoke. All patients with thromboangiitis obliterans regardless of age or circumstances should be urged to stop smoking, as should patients with arteriosclerosis obliterans. There will be some exceptions to this, such as the 85-year-old man who has a minimal amount of difficulty and has a nonprogressing disease, but in general most patients with arteriosclerosis obliterans would be better off if they stopped the use of tobacco. Finally, all patients should stop if they have significant arterial insufficiency at rest in which there is danger of losing the extremity.

Does anyone have anything to add?

MONTGOMERY: You mentioned veins a while ago but have not included mention of them in your summary.

HINES: I do not think we have enough evidence to insist that patients with venous insufficiency also stop smoking.

GOETZ: May I have your opinion on how you would answer this question, which I am frequently asked? I know how I would answer it. Every now and then a patient will say, "But I do not inhale. Can I smoke, provided I do not inhale?"

HINES: In the first place I do not think we can take the patient's word as to whether or not he inhales. It would be necessary to show that by some test. No, I should not make any exception on the basis of whether the patient thought he inhaled.

GOETZ: I wanted to get this confirmed, because much less nicotine is being absorbed in the mouth and the patient will probably have this information from his reading.

HINES: If someone can show by statistical study that the noninhalers get into less trouble than inhalers, it would be useful, but I do not think we have that information.

I propose now to pass on to discussing some specific aids to patients included in the groups advised to stop smoking, and consider them from two standpoints: first, deterrents to the effect of nicotine on the blood vessels, and, second, deterrents to the tobacco-use habit.

We have heard some interesting things about the effect of reserpine during this conference. Could reserpine be used as a deterrent to the effects of nicotine on the blood vessels?

WOODS: It is my impression from experiments, admittedly directed toward another problem, that oral reserpine in doses used in humans for hypertension does not alter vasomotor response to smoking very much.

MONTGOMERY: It seems to me pretty clear that, in so far as the peripheral vessels are concerned, the local release of vasopressive material is not the main problem. For about 10 or more years there have been good studies of the effect of sympathectomy in abolishing or certainly greatly decreasing the action of nicotine. Most of the nicotine effect in the periphery appears to be by way of the sympathetic nervous system.

WOOD: I had meant to imply that.

GOETZ: I understood Burn to say in his paper that he thought the failure to get vasoconstriction in sympathectomized limbs was due to this very effect. Burn reported a decrease in catechol amines following sympathectomy.

ROTH: The patients we are studying have not had any reserpine, only sympathectomy. As I showed in my paper there was no vasoconstriction in the feet that had been sympathectomized; we have numerous similar cases.

HINES: The value of this discussion is largely theoretical because I do not think anyone has done a good clinical study on the effects of reserpine on the various conditions. It has been reported that reserpine is beneficial in patients who have Raynaud's disease. I have not found it to be so in patients who have Raynaud's disease with severe and progressing symptoms. It may be beneficial in mild cases that do not need treatment other than protection from cold.

Reserpine does not significantly reduce the blood pressure-raising effect of the cold pressor test in patients with hypertension.

If a patient has sympathectomy, can he continue to smoke?

GOETZ: Obviously he cannot continue to smoke. When we do a sympathectomy, we do it on a patient with a systemic disease. We usually do the sympathectomy for disease in the lower or upper limbs, knowing full well that the patient also has arteriosclerosis of the coronary and other vessels. Because we are concerned with a systemic disease, I am in favor of telling the patient to stop smoking after he has had a sympathectomy even if there is no longer a reduction of the blood flow to the extremities on smoking.

HINES: I think the question is important because there are some surgeons who are telling patients, when they have a successful bypass operation for occlusion and thus restore the circulation to normal, that it is no longer necessary for them to stop the use of tobacco. Will Goetz comment about that?

GOETZ: In my experience, many a patient would rather have a bypass operation than stop smoking. This is unfortunately the psychological situation in which we find our patients. If we do a bypass operation, we shall be able to improve the circulation in that particular area but we shall do nothing to change the natural course of the disease in other areas. The patient has, of course, arterial disease not only in the periphery, and therefore he must stop smoking.

HINES: Can we be told about the vasodilating effects of alcohol counteracting the vasoconstricting effects of tobacco?

ROTH: In our studies we gave one ounce of ethyl alcohol on an empty stomach, which produces good vasodilation. We explored the effects of smoking from 10 min. after the giving of the alcohol for an hour and one half, and there was no time when the smoking did not result in a marked vasoconstriction.

As I said in my paper, it may be possible to take enough alcohol to counteract the effects of smoking, but one ounce of 95 per cent ethyl alcohol before breakfast is really a good dose.

The patient who has arteriosclerosis and does not smoke may have some relief and a little vasodilation from the alcohol, but I do not think that it will protect him during smoking.

HINES: May I spend the remaining few minutes on suggestions that may help the patient to stop the use of tobacco and also may show how a physician can help the patient to stop the use of tobacco when it is important for him to do so.

I ask Roth another question. What about drugs and other procedures that are advertised in newspapers and magazines as guaranteed to make it more pleasant to stop than to continue smoking?

ROTH: I have watched patients try all the suggestions for the cessation of smoking, and I think each individual has to work out his own method. One method is to take tranquilizers; some people have done very well with that. Most magazines have some method for the cessation of smoking. I think a person must make up his mind that he wants to stop smoking. That is the one thing that gives enough courage to go on. I haven't seen anything else that stops it.

Lobeline is sometimes used. This drug, and any of the others that I have seen used for the cessation of smoking, have not been very effective. Possibly someone knows of something else?

HINES: We may as well recognize that it is very difficult for a heavy smoker to stop smoking, and we should follow some pretty definite rules or principles in trying to aid him.

In the first place, it is necessary to be very positive in talking to the patient. Do not quibble—this is no time to listen to the wee small voice of the statistician saying there is no proof of this. It is necessary to talk positively to the patient although proof of what the patient is being told is not 100 per cent. If any little thing is let slip that allows the patient to think that it is not absolutely essential for him to stop smoking, he will use it as an excuse to continue smoking. I have never known a patient who could stop smoking gradually. And I do not think patients can be persuaded to stop smoking unless they are given a very strong incentive to do so. The only incentive in most of the situations we have talked about that is strong enough is the incentive of keeping or losing his legs. This does impress the patient and you should tell him right out that he will have to decide whether or not he wants to continue to chew or use tobacco or lose his legs.

Does anybody else have anything they tell a patient that might be helpful to persuade him to stop smoking? What about some of the smokers here? Do they know of any incentives that have not been mentioned that might persuade them to stop smoking?

MONTGOMERY: What do you tell a patient who stops smoking and then loses his legs?

HINES: Well, I hope not to see him. I hope he goes to Philadelphia.

MONTGOMERY: We do see such patients, and it is interesting that they do not appear in your statistics.

HINES: That is right; we did not have any of them in our series. When we get enough such patients we shall, of course, see them.

References

1. WOOD, J. E., J. R. ESTERLY & V. A. MCKUSICK. 1960. Blood flow in Milroy's disease. Clin. Research. **8**(2): 195.
2. SILBERT, S. 1945. Etiology of thromboangiitis obliterans. J. Am. Med. Assoc. Suppl. I. : 5–9.
3. GIFFORD, R. W., JR. & E. A. HINES, JR. 1951. Complete clinical remission in thromboangiitis obliterans during abstinence from tobacco: a report of a case. Proc. Staff Meeting Mayo Clinic, Rochester, Minn., June 20. : 241–245.
4. JUERGENS, J. L., M. W. BARKER & E. A. HINES, JR. 1960. Arteriosclerosis obliterans: a review of 520 cases. Circulation. **21**: 188–195.
5. JOHNSTON, LENNOX. 1957. The disease of tobacco smoking and its cure. Christopher Johnson. London, England.

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